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THE SITE OF THE FORMATION OF BILIRUBIN

FRANK C. MANN, CHARLES SHEARD, JESSE L. BOLLMAN AND EDWARD J. BALDES

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, and Section on Physics, Mayo Clinic, Rochester, Minnesota

Received for publication July 31, 1925

In work previously reported (Mann, Bollman and Magath, 1924), data were presented proving that bilirubin is formed in the body even after the surgical removal of all the intra-abdominal organs, thus conclusively demonstrating that there is an extra-abdominal source of bilirubin and that neither the liver nor any other of the intra-abdominal organs is necessary for its formation. The employment of the spectrophotometer for the identification and measurement of the bilirubin in the plasma showed that the colorless plasma of the normal dog contains a small but measurable amount of bilirubin, although not enough to be identified by any other method (Mann, Sheard and Bollman, 1925). These two facts: first, that bilirubin is formed by some tissue outside the abdomen, and second, that by means of the spectrophotometer bilirubin may be detected in the colorless plasma of the normal dog, furnished the basis for the present investigation.

Since bilirubin accumulated progressively in the body after the liver had been eliminated, it was clear that some organ or tissue, other than the liver, was pouring the pigment into the circulation. The first step in an attempt to locate the site of the formation of bilirubin was to determine whether there was any measurable difference in the bilirubin content of the arterial and venous blood of any organ or other large vascular area. The questionable value of an attempt to determine the action of an organ with regard to any specific substance by an estimation of the amount of the substance entering and leaving the organ has often been recognized. The change, if there was a change, in relation to the volume of blood flow, has usually been too slight to be measured by existing methods. However, the spectrophotometer offered a very exact method of identification and determination of variations of minute amounts of bilirubin and made it

feasible to attempt to determine more definitely the site of extrahepatic bilirubin formation.

Since it had been shown that bilirubin is formed extra-abdominally, it seemed best to determine first whether there was any difference in the bilirubin content of the arterial and venous blood of the large vessels outside the abdomen. Accordingly, an examination and comparison of the bilirubin content of the blood of the carotid arteries and jugular veins, and of the femoral arteries and femoral veins, was made.

The spectrophotometer yielded results far more conclusive than had been anticipated. There was usually a small but definitely greater amount of bilirubin in the blood returning from the head and hind limbs than in the blood going to these structures. It was therefore advisable to learn whether this was true of all venous blood or only of that in certain areas. If the former, it would prove a very general distribution of the bilirubin-forming tissue, whereas if the latter, it would show that bilirubin was formed in a greatly restricted area. Accordingly, we compared the bilirubin content of all the major arteries with that of their accompanying veins.

The bilirubin content of the blood returning from the kidneys and intestine was the same as that of the blood going to these organs. There was an increase in the bilirubin content of the blood returning from the spleen, limbs, thorax and head. Blood obtained from all the various large arteries of the body contained approximately the same amount of bilirubin. It is obvious, since bilirubin is excreted by the liver, that data concerning the comparison of the bilirubin content of the blood going to and returning from the liver would not be of any value with reference to whether or not bilirubin was formed in the organ, and therefore the hepatic blood was not examined.

The venous blood which contained a greater amount of bilirubin than the arterial blood drained mainly from four kinds of tissue: spleen, skin and fascia, muscle, and bone marrow. Since the spleen has a separate circulation, it was a simple matter to obtain blood from that organ alone. It was not easy, however, to determine what tissue or tissues were responsible for the bilirubin in the systemic veins, although it was possible to obtain blood separately from the skin and muscle, and bone marrow. When the bone was removed from a hind limb and the circulation maintained to the muscle, skin and fascia, the difference in the bilirubin content of the venous and arterial blood of the limb disappeared, and when the muscle and skin were removed, the blood flow to the bone marrow remaining intact, the bilirubin content of the venous blood from the bone marrow was greater than the arterial blood. Venous blood obtained directly from muscle and skin had the same concentration of bilirubin as arterial blood.

It was thus evident that bilirubin was added to the blood stream in the bone marrow and spleen, and that bilirubin was formed in these tissues.

The results of this investigation are definite and can be simply stated, but various difficulties were encountered. These were mainly in: *1*, obtaining blood specimens separately from the necessary sources; *2*, preventing any hemolysis; and *3*, obtaining a perfectly clear solution for examination.

Method of obtaining specimens of blood. All the experiments were performed on dogs, and all operative procedures were done under ether anesthesia. Because of the large amount of blood required, large animals were used. The specimens from the major arteries and veins were obtained by means of a syringe after direct puncture of the vessel. In order not to alter the activity of the part by a change in blood flow before obtaining a sample of the venous blood, the latter was always obtained first. The blood draining from the bone marrow only was obtained from the tibia by a modification of the excellent method developed by Drinker (1922). Briefly, this consisted in sectioning and retracting all the muscles of the leg and amputating the foot. All the blood vessels from the knee distally were ligated except those to the tibia. When the circulation from the tibia had thus been completely isolated, a cannula was inserted into the vein at a point where it was of a suitable size, usually at the popliteal space, and the blood allowed to flow directly into the centrifuge tubes. It should be emphasized that in these preparations the blood supply to the tibia was completely isolated so that the blood obtained had circulated through this structure only. In all experiments, after the specimens were obtained, the artery to the tibia was injected with India ink or gentian violet, and the exact vascular area which the venous specimen had traversed was thus determined. The specimens which had circulated through muscles only were obtained by isolating the gastrocnemius muscle from all connections except its artery and vein, and then inserting a cannula into the latter, the blood flowing directly into the centrifuge tube. The one precaution necessary in obtaining the specimens from the spleen was to ligate all vasa brevia.

Physical methods and procedures. After exposure of the arteries and veins, samples of blood were withdrawn into dry syringes with the aid of small needles, or bled directly into the centrifuge tubes. About 30 cc. of blood were usually sufficient. The samples were immediately transferred to dry centrifuge tubes and allowed to clot. The clot was then loosened from the wall of the tube and centrifugalized at high speed. Ten cubic centimeters of the serum were placed in a 50 cc. centrifuge tube containing 15 cc. of 95 per cent alcohol and 5 cc. of acetone. The tube was then stoppered and shaken well and placed in the ice box for two hours. After

again centrifugalizing at high speed, the clear supernatant liquid was pipetted into a dry tube for examination with the spectrophotometer.

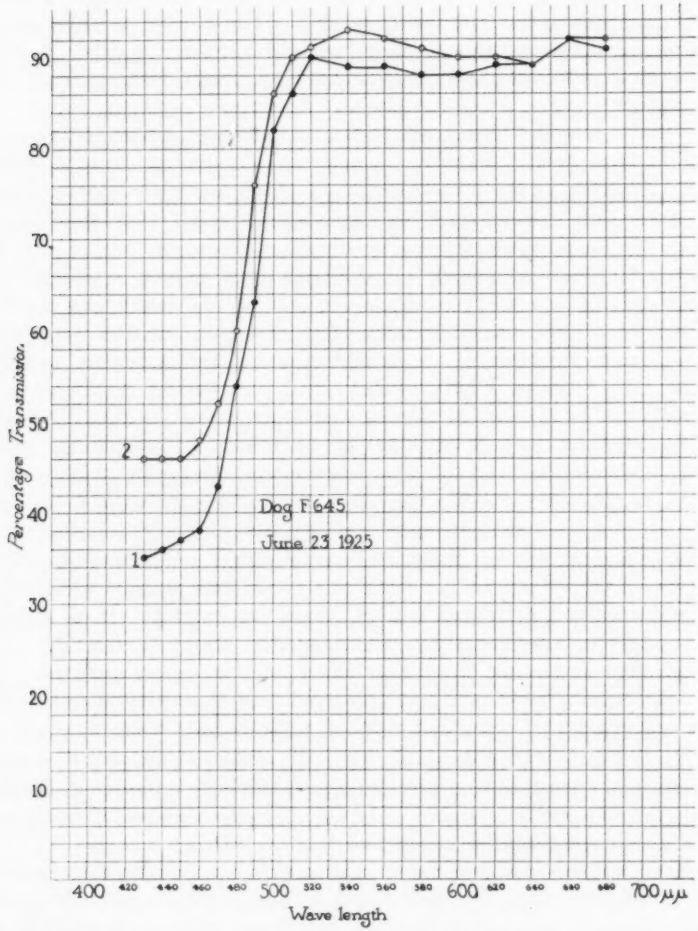

Fig. 1. Curves showing greater bilirubin concentration in the blood of the jugular vein than of the carotid artery. Curve *1*, light transmission curve of alcoholic extract of serum from the right jugular vein. Curve *2*, light transmission curve of alcoholic extract of serum from the right carotid artery.

The spectrophotometer used in the investigations was one recently brought out by Keuffel and Esser, referred to in the literature as a "color analyzer." It consists essentially of a lamp-house carrying two magnesium

carbonate blocks (cut from the same cake) at the rear; these blocks serve as sources of light. The beams of light reflected by the blocks, after passing through two suitable openings in the front of the lamp-house,

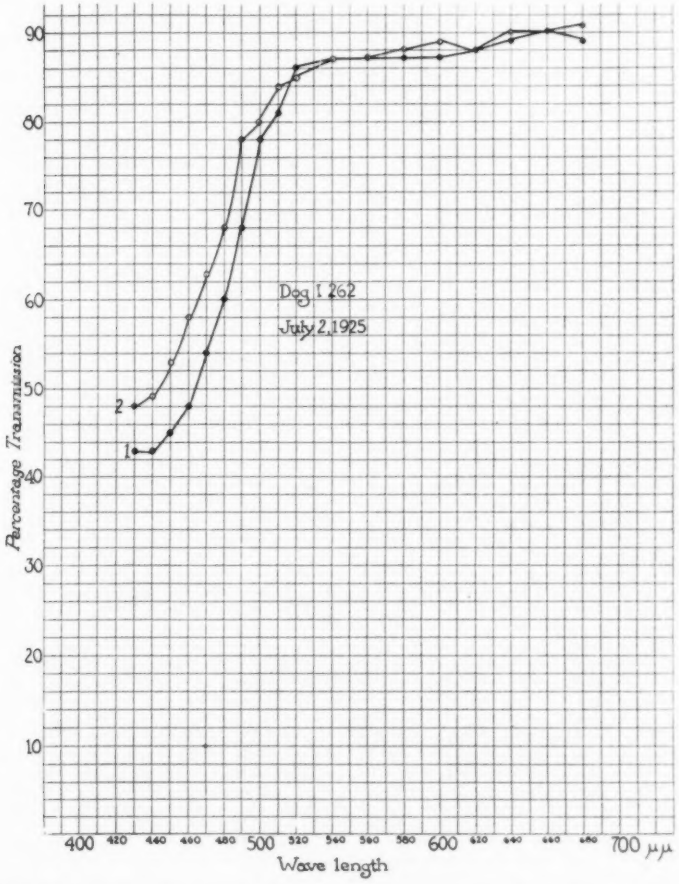

Fig. 2. Curves showing greater bilirubin concentration in the blood of the axillary vein than of the axillary artery. Curve *1*, light transmission curve of alcoholic extract of serum from the right axillary vein. Curve *2*, light transmission curve of alcoholic extract of serum from the right axillary artery.

enter the two receptacles containing the solution and the solvent respectively, which are placed in the proper position in front of the slit of the spectrometer. The spectrometer proper does not differ in fundamentals

from the ordinary constant-deviation type of instrument except for a biprism, placed in front of the telescopic lens system, and an observing

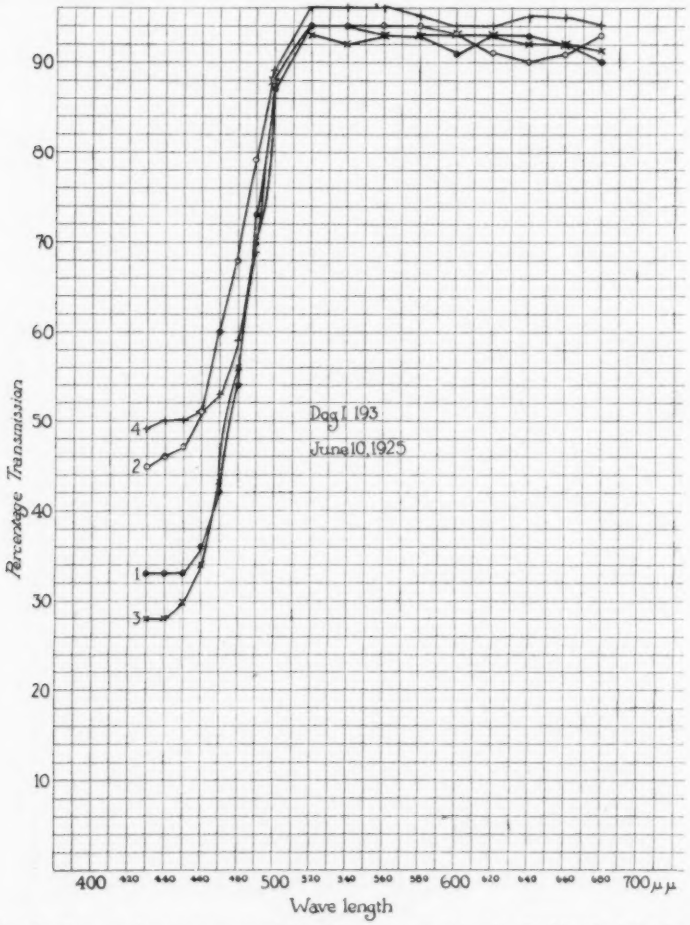

Fig. 3. Curves showing greater bilirubin concentration in the blood of the femoral vein than of the femoral artery. Curves *1* and *3*, light transmission curves of alcoholic extracts of serum *1*, from the right, and *3*, from the left femoral veins. Curves *2* and *4*, light transmission curves of alcoholic extracts of serum: *2*, from the right, and *4*, from the left femoral arteries.

(exit) slit in the eyepiece. A balance in the intensities of the light transmitted by the standard cell and by the solution under examination was made by means of a sector photometer, the intensity of the light trans-

mitted by the standard cell only being varied. In all the experiments described here, both containing tubes were 10 cm. in length. The standard cell was filled with distilled water, the other carried the solution to be

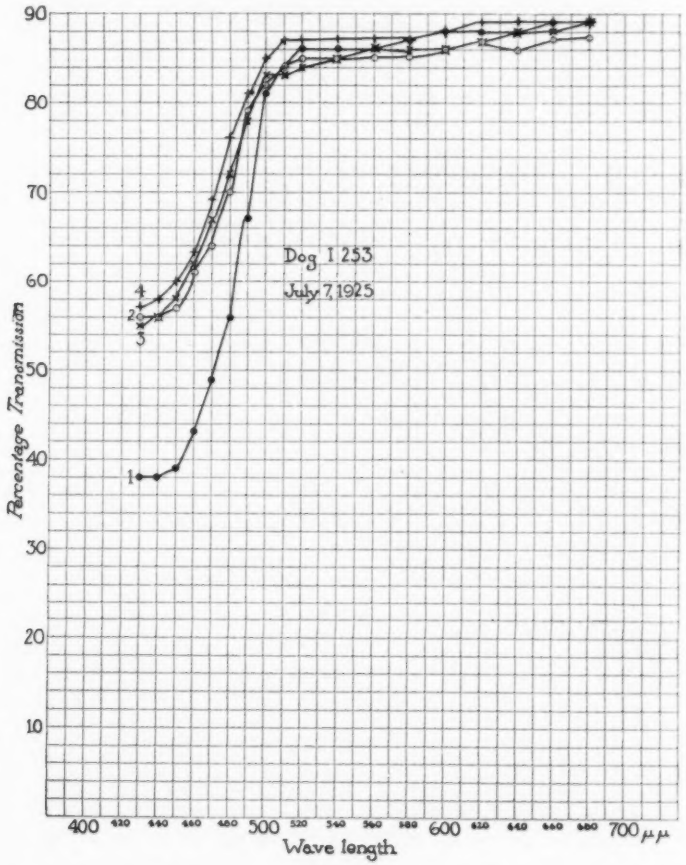

Fig. 4. Curves showing greater bilirubin concentration in the blood of the splenic vein than of the splenic and renal arteries or renal vein. Curve *1*, light transmission curve of alcoholic extract of serum from the splenic vein. Curves *2*, *3* and *4*, light transmission curves of alcoholic extracts of serum: *2*, from the splenic artery, *3*, from the left renal vein, and *4*, from the left renal artery.

examined. This procedure was adopted in order to introduce equal quantities of glass and liquid into the two optical paths.

The method of getting the data shown in the curves of figures 1 to 6 is as follows: White light is passed through the two containers placed in front

of the housing carrying the rotating sectors and admitted to the spectrometer. The spectrometer is set at any desired wave length by means of a

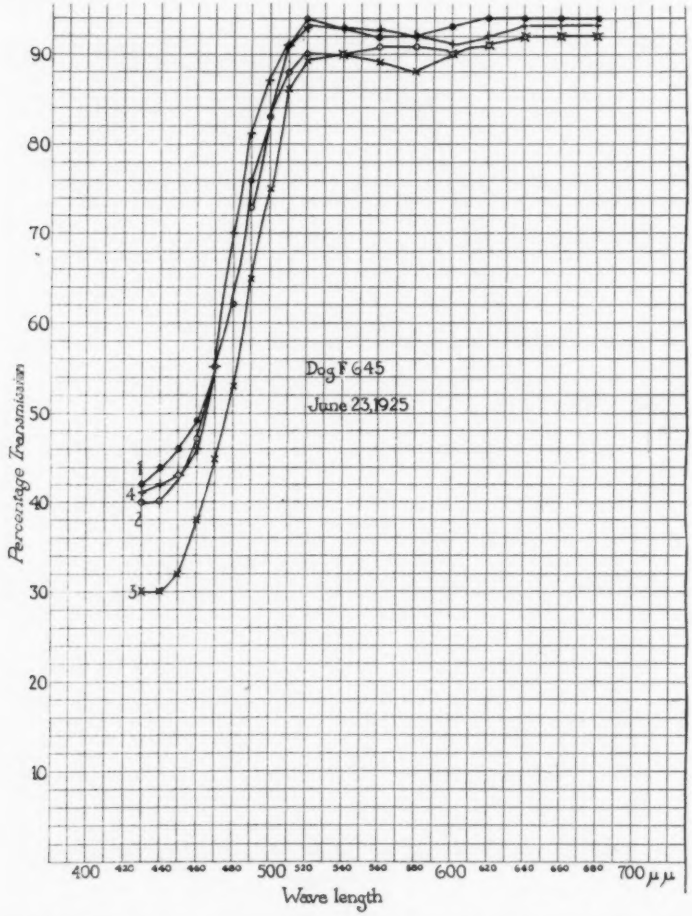

Fig. 5. Curves showing the greater bilirubin content of the blood of the femoral vein than of the femoral arteries or the femoral vein after removal of the bone from the leg. Curve *3*, light transmission curve of alcoholic extracts of serum from the left femoral vein. Curves *1*, *2* and *4*, light transmission curves of alcoholic extracts of serum: *4*, from the left femoral artery, *2*, from the right femoral artery, and *1*, from the right femoral vein after removal of the bone of the right leg.

calibrated wheel. As an illustration, with a setting of 660 millimicrons, which is in the red region of the spectrum, the observer looking through the

eye-slit sees two small semi-circular colored areas in juxtaposition, with the dividing line horizontal. Both halves of the circle will have the

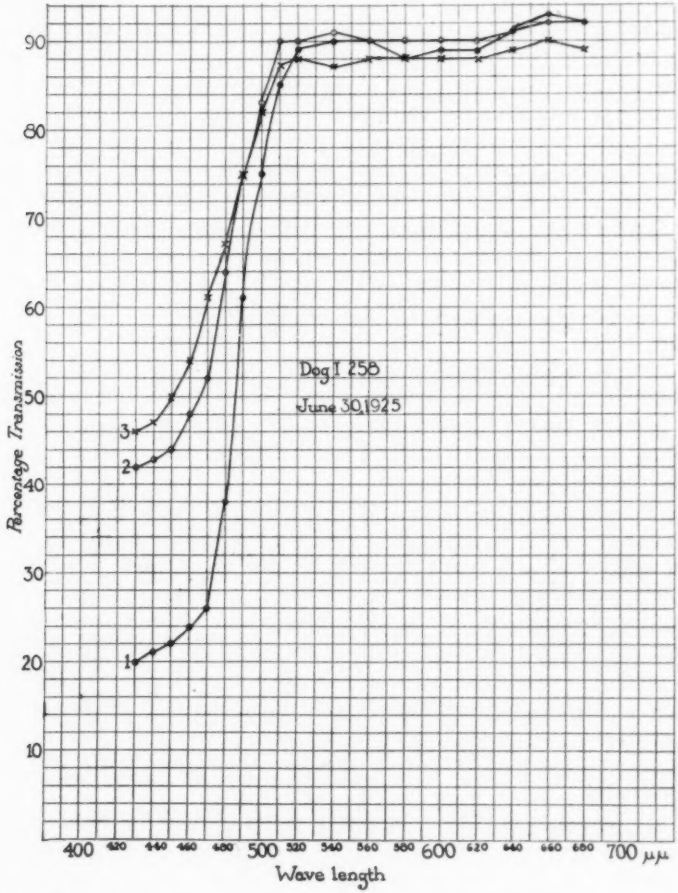

Fig. 6. Curves showing the greater bilirubin content of blood returning from bone marrow as compared with blood returning from muscle, and arterial blood. Curve *1*, light transmission curve of alcoholic extract of serum from blood returning from the right tibia only. Curves *2* and *3*, light transmission curves of alcoholic extracts of serum: *2*, from blood returning from the right gastrocnemius muscle only, and *3*, from the right femoral artery.

same hue (red), but not necessarily the same brightness or intensity. The equality of brightness adjustment or match is then made by varying the size of the sector opening in the two rotating sectors placed in front of the

spectrometer. The percentage transmission of the solution as compared to the percentage transmission of the liquid (water) in the so-called standard cell is read directly from the calibrated drum-head. This drum-head is mechanically attached to the sectored disks in such a manner as to permit rapid turning of it by hand, thus providing a quick way of varying the relative proportions of open and closed sector areas. In determining the percentage transmission for any given wave length, it has been our custom to allow a few moments for the adjustment of the eye to the spectral hue presented, and to record as the final reading, for each wave length taken, the average of from five to ten separate determinations which do not vary more than one or two points, respectively. After having made the measurement for equality of brightness for any given wave length, the procedure, as outlined, is repeated for as many determinations, and for as many spectral regions or wave lengths as are deemed necessary.

Experimental criteria in spectrophotometric measurements. Much has been learned in these investigations concerning the criteria which had to be adopted and followed in order that the spectrophotometric data as regards substances in blood serum, especially bilirubin, might be used in deducing final conclusions. These criteria are clearness of the solution, and its freedom from hemoglobin.

If solutions are turbid or carry fine particles in suspension, the general type of curve shown in our illustrations will not be obtained. On account of the scattering and absorption of light due to particles in suspension, there is a marked increase in the percentage of light absorbed in the general absorption zone of from 500 to 430 millimicrons. Hence, it is impossible to draw any valid conclusions with reference to the quantity of bile pigment present in various serums from any given animal, unless all the serums examined in the same series are of nearly equal clarity. We have adopted as a general criterion on this point a spectrophotometric reading of the order of 90 per cent or better through the region from 700 to 600 millimicrons.

It was found that plasma or serum could seldom be obtained clear enough for direct use in the spectrophotometer, and neither was dilution of these with sodium chlorid solution satisfactory. Accordingly, various other methods of obtaining bilirubin in solution from plasma or serum were tried, the most satisfactory of which was precipitation with alcohol and acetone, as outlined. Crystal-clear solutions of bilirubin may be obtained in this manner, provided the solutions used are kept at a low temperature (10° to 15°) and care is taken to avoid bringing the clarified solution in contact with moisture.

That the solution be free from hemolysis is equally as important as that it be clear. We have found that if we put a single drop of hemolyzed blood in a tube 10 cm. in length, containing a serum clear enough to give a

curve similar to those shown in figures 1 to 6, we obtain spectrophotometric values indicating a marked absorption region, with a maximal absorption value of about 80 per cent, from 590 to 560 millimicrons, together with an increased reduction in light transmission in the general absorption zone characteristic of bilirubin (from 500 to 430 millimicrons) of approximately 40 per cent. It is evident that accurate data concerning the concentration of bilirubin cannot be obtained in the presence of amounts of hemoglobin sufficient to cause light absorption in this zone. As a standard, therefore, we have adopted the use of only such spectrophotometric data as show less than 10 per cent absorption in the region of from 580 to 560 millimicrons.

We know of no methods that are comparable to spectrophotometric measurements as an aid in the elimination of the two sources of error we have cited. Furthermore, we wish to emphasize our belief that conclusions drawn from data obtained in researches on bile pigment, conducted by the spectrophotometric method in which no standards for clarity and freedom from hemolysis are set up and maintained, are open to grave doubt.

A point of interest is that it is possible, with the unaided eye, to make approximate comparisons of the amounts of bile pigment in various serums contained in tubes of the same length, for example, 10 cm. That is, it is possible to compare simultaneously with the naked eye the depth of color (generally a light amber) in any two given tubes, and thereby rapidly obtain an estimate of the relative amounts of bile pigment in each. However, such comparisons with the naked eye are of no value unless spectrophotometric determinations have indicated a clarity of at least 90 per cent in the long visual wave-length region, and freedom from hemolysis (less than 10 per cent).

Other optical methods of estimating bile pigment. We have checked several of our spectrophotometric curves, as shown in figures 1 to 6, with spectrograms taken with a Bausch and Lomb constant-deviation spectrograph. These spectrograms, through a determination of the wave-length density relations, substantiate the conclusions reached spectrophotometrically. We have, in one or two instances, checked our findings as made by the Keuffel and Esser spectrophotometer with a photometer of the Martens polarization type. We prefer the experimental methods which we have followed because of the rapidity, ease, and fair accuracy in getting readings with them. Since we are interested solely in getting differences in the absorptions of various blood serums in the region from 500 to 430 millimicrons, the probable percentage inaccuracy of readings made by the method pursued, which is of the order of one to two points at the most, is of no great moment.

Comparison of sensitiveness of spectrophotometric method with the van den

Bergh method. In order to get some idea of the sensitiveness of the spectrophotometric estimations as compared with the van den Bergh, a sample of bile was taken from the gall bladder of a normal dog, and diluted to approximately 0.1 mgm. bilirubin for each 100 cc., as determined by the van den Bergh method. This is approximately the limit of accuracy of the van den Bergh method. On repeated dilution with alcohol, it was possible to determine quite accurately by the spectrophotometric method the presence of one-fiftieth the smallest amount measurable by the van den Bergh method. Comparing the spectrophotometric determinations with known dilutions of bile, it was found that quantities of bile pigment were being dealt with, varying from 0.04 to 0.015 mgm. for each 100 cc. Further tests on the sensitiveness of the spectrophotometric estimation of bilirubin are now being made by Sheard and Baldes.

Discussion. It was not always easy to obtain specimens of blood that had traversed only the desired tissue. Usually there was little difficulty in securing specimens from the vessels of organs having their own definite circulation, such as the kidney, spleen, and intestine. It was also easy to obtain specimens from the head and limbs. Although it was sometimes difficult to isolate the blood supply of bone marrow and muscle, it was always possible to prove, by the injection of India ink, when isolation had been accomplished.

Early in the investigation it was seen that it was very important to prevent even the slightest hemolysis, and our technic was accordingly improved with that end in view. However, the spectrophotometer so clearly indicated even a slight trace of hemoglobin in a specimen that such a factor was never a source of error in our deductions. To the naked eye such a specimen might appear to be clear. It is again emphasized that when hemoglobin was present, the specimen was discarded.

The development of turbidity in the specimen prepared for examination was also a source of trouble. This difficulty could usually be eliminated by keeping the specimen cold. Again, however, turbidity was not a source of error in our positive results because the spectrophotometer definitely indicated when the solution was not clear and all specimens that failed to clear were discarded.

While all conclusions are based on the results obtained by the spectrophotometer, it was found very early that a difference in the serum of the various specimens could often be detected with the naked eye. It was frequently possible to note with the naked eye a difference in color between the serum of the blood from the femoral vein and artery, and that from the splenic vein and artery. However, as stated, the deduction that this difference in color was due to a difference in the bilirubin content could only be made after a spectroscopic examination which eliminated the possibility of hemoglobin and turbidity.

The results of the experiments show conclusively that bilirubin is added to the blood as it traverses the spleen and bone marrow. Bilirubin was not added to the blood while passing through any of the other tissues studied, which included practically all the tissues in the body except the liver. The amount of bilirubin detected in these tissues was so small that it is very evident that bilirubin was not being formed in them. Whether or not bilirubin is formed in the liver has not been determined. In any event, the spleen and bone marrow are definitely proved sites of the formation of bilirubin.

It is not necessary to review here the extensive literature on the site of the formation of bilirubin. An excellent and complete review of the subject has recently been presented by Rich (1925), who has also reviewed with Rienhoff the work on the bilirubin content of the splenic artery and vein (Rich and Rienhoff, 1925). Aschoff (1924) has discussed in detail certain phases of the problem of the site of such formation, as well as the reticulo-endothelial system. In this connection we wish to emphasize that the differences we obtained in the bilirubin content of the different serums could not have been detected by any other known method than the one we employed.

Our results do not necessarily prove conclusively that the reticulo-endothelial system is the site of the formation of bilirubin as enunciated by McNee (1913, 1923) and Aschoff (1924), but they do prove beyond any doubt that bilirubin is formed in two of the sites where the reticulo-endothelial cells are most abundant. This is strong evidence that the theory that bilirubin is formed by the cells of the reticulo-endothelial system is correct, and that the cells of the same type in the liver can also form bilirubin. The cells forming bilirubin would thus be rather widely distributed and as a tissue be located mainly in the spleen, bone marrow and liver, although occurring in other places, such as the lymph-nodes. It is highly probable that this definitely proved, rather wide distribution of the cells capable of forming bilirubin will correlate many of the seeming contradictions, divergent experimental, and clinical observations on the formation of bile pigment and jaundice.

In this connection it might be pointed out that in all probability there is normally the same relatively large amount of tissue reserve in this bilirubin forming tissue as we know there is in other tissues. That is, the bilirubin-forming tissue greatly exceeds that for the ordinary needs of the body. It is also probably true that only a small part of this tissue is active at any one given time. Furthermore, since the bilirubin-forming cells are scattered in widely different kinds of tissue, it is possible that the number of the cells in any given location varies greatly in different individuals. It could also be possible that those bilirubin-forming cells located in the spleen where they had access to a very generous blood supply and where

their product would pass directly to the liver to be excreted, would respond to a lower threshold of hemoglobin than the same type of cell in other parts of the body. If the foregoing conjectures are true, it would explain many uncorrelated facts, for instance, the variations in the effect of splenectomy on changes in the liver, lymph nodes and bone marrow; variability in the rate of the formation of bilirubin; various types of jaundice, and so forth.

SUMMARY

A comparison of the bilirubin content, as measured by the spectrophotometer, was made of the arterial and venous blood of most of the large organs of the body and other isolated vascular areas. In most of the vascular areas, the bilirubin content of specimens of blood from the two systems was the same, but the bilirubin content of the venous blood was greater than that of the arterial blood of spleen and bone marrow, which proves that bilirubin is formed in the spleen and bone marrow.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF LIGHT

II. Depression of Arterial Blood Pressure[1]

C. I. REED[2]

From the Hull Physiological Laboratory of the University of Chicago

Received for publication July 30, 1925

In a previous communication were reported (Reed, 1923) the results of exposure of the eyes of dogs anesthetized with *chloretone* to light of approximately 100 f.c. intensity from a 1000 wtt. nitrogen tungsten projection lantern. The predominant effect of brief exposures was a transient depression of blood pressure which was thought at that time to be a purely nervous effect. In a subsequent communication (Reed, 1925a) were reported further experiments of the same nature on dogs under *ether* anesthesia; it is the purpose of this paper to present a more detailed discussion of the results of the latter experiments.

Depression of blood pressure under the influence of light from various sources is not a new observation. Levy (1916), Gassul (1920, 1924), Rollier (1923), Reyn (1924), Hall (1924), Hasselbalch and Jacobaus (1907), Meyer (1922), O'Elsnitz (1913), Orum (1906), Rothman (1923), Rother (1924), Haussmann (1923), Austgen (1919), Finsen (1901), Kimmerle (1921), Löwenhart (1918), have all observed depression of general arterial blood pressure under the influence of light in some form of irradiation. Some of these reports are based on experimental investigations, others on clinical observations. However, Young, Breinl, Harris and Osborne (1920) and Aimes (1925) claim that under certain conditions blood pressure may be elevated by irradiation, while Günther (1923) finds a short rise followed by a long progressive fall.

Schroeder (1924) and others have observed the same results after roentgen radiation, chiefly in cases of hypertension.

Most observers have ascribed this depression in blood pressure to dilatation of cutaneous vessels in the production of erythema. However, there is some disagreement on this point. Gassul (1920) has claimed that he has been able to show experimentally that the visceral blood vessels in rats are greatly dilated after irradiation.

[1] A small part of the experimental work was done at the University of Kansas under a grant from the Graduate Research Committee.

[2] National Research Council Fellow.

Since chloretone did not prove to be an entirely satisfactory anesthetic for the purpose, it was decided to resort to ether administered by tracheotomy. As a control on blood pressure changes under ether anesthesia a series of 12 experiments was undertaken. Blood pressure was recorded from the carotid artery. After quick induction of anesthesia and the necessary operations were performed, the animals were allowed to remain undisturbed in a stage of anesthesia in which the corneal reflex was just apparent on light touch. A stabilized level of blood pressure as occurring after all other conditions were stabilized was taken as a 100 per cent level. The pressure was measured at five minute intervals for a period of at least

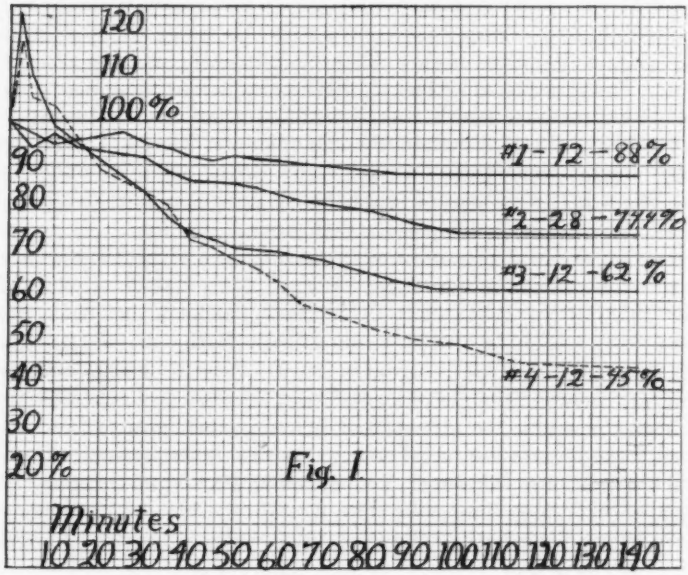


Fig. 1

two hours for each experiment and the results condensed into a composite curve shown in figure 1 (curve *1*).

For the period in question there was an average depression from the initial level of only 12 per cent. There are some minor irregularities in the curve which reached a stabilized level at the end of one and one-half hours. The greatest depression in any one experiment was 23 per cent; at the other extreme there was in only one case a rise in blood pressure of 5 per cent. This one case is not easily accounted for but is included in order to adhere strictly to results as obtained.

In series II (fig. 1, curve *2*), 28 experiments are included in which one

eye of the anesthetized animal was exposed, after blood pressure became stabilized, to a beam of light from the projection lantern as described above. All other conditions were maintained as in the first series. The total average depression in this series was 25 per cent, this level being reached at the end of one hour and forty minutes. The maximum in any one experiment was 52 −per cent, the other extreme being again a 4 + per cent rise in pressure.

The composite curve shows much the same type and degree of irregularity as curve *1*, but most of the individual experiments showed great irregularity after the light had been applied for several minutes. In half of the experiments there was a rise in pressure at some stage in the first 40 minutes of irradiation, sometimes slightly above the initial level and usually sustained for from 5 to 15 minutes. There was, however, another type of irregularity, a quick vasomotor change, sometimes pressor in effect, sometimes depressor, often amounting to from 10 to 20 mm. Hg and lasting only a few seconds. To be sure, this sort of change is occasionally seen under anesthesia when no experimental manipulations are employed but it became very common in irradiation of the eye. These two types of changes are probably not specific but are more probably of reflex origin.

In several experiments there was evidence of vagal disturbance since there was often a sharp drop in pressure extending over a period of three or four heart beats, very like the response to vagal stimulation with strong single induction shocks. These would sometimes occur singly and at long intervals, in other cases as frequently as 20 times a minute.

These results suggested that the vagus was involved as a pathway by which impulses from the eye, strongly irritated, might induce a general fall in blood pressure. In preparation for testing this possibility series III (fig. 1, curve *3*) was undertaken, consisting of 12 experiments in which a simultaneous bilateral vagotomy was performed as soon as blood pressure became stabilized (Reed, 1925b). The animals were then treated as in series I. The results are shown in a composite curve. The total average depression was 38 per cent, maximum 80 per cent, minimum 20 per cent.

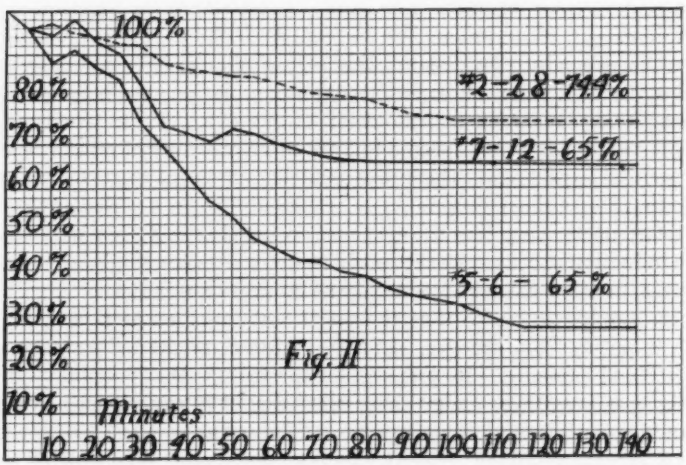
In series IV (fig. 1, curve *4*) are included 12 experiments in which, after bilateral vagotomy, the light was applied to one eye as in series II. The composite curve shows a total depression of 55 per cent, maximum 82 per cent, minimum 25 per cent. The range is practically the same but the average result is much greater than in series III.

The fact that these two curves practically coincided for 45 minutes; that cutting the vagi did not abolish the effect; furthermore, that vagisection did not abolish or prevent the vasomotor and cardiac irregularities above mentioned as occurring in series II, indicated that the results of irradiation were not wholly of nervous origin.

It was now decided to employ a carbon arc as a source of light. The

beam from this source was condensed with a heavy glass lens so that it was possible to get 1250 f.c. intensity on the eye. A spectogram of this beam showed a sharp filtration of ultra-violet at 3300 Ångström units. Series V comprised 6 dogs of which one eye was exposed to this beam for approximately two hours. The composite curve is shown in figure 2 (curve 5). The average depression of blood pressure was 71 + per cent, maximum 75 per cent, minimum 61 per cent. It was subsequently determined that the amount of depression was roughly proportional to the intensity of the light up to about the intensity used here. Greater intensity of light produced more rapid results but of no greater magnitude.

With series V as a control group, series VII was now run through (fig. 2, curve 7), consisting of 12 animals in which the light from the carbon arc



was employed as in series V but only after the optic nerve had been cut. The technic for this operation consisted of cutting a narrow slit in the conjunctiva at the outer canthus, locating the optic nerve with a curved probe and crushing it with a pair of long, narrow pointed hemostats. Postmortem examination confirmed the result of the operation in every case included. The nerve trunk was entirely crushed in two. In control dogs blood pressure was not depressed beyond that of animals in series I, as a result of this operation.

The other eye was now covered and the denervated eye was irradiated with the beam from the arc. The composite curve (no. 7) is shown in figure 2. The average depression in blood pressure now amounted to only 35 per cent; maximum 62 per cent, minimum 80 per cent. The total effect

is thus slightly less than half that obtained with intact eyes. After the first half-hour there was sharp divergence of the two curves.

While this difference indicates that a nervous factor *may* play some part in the final result, it must also be borne in mind that the operation interfered with the blood supply, especially the central artery of the retina so that this factor must be taken into account in making any judgment of these results.

In view of all the evidence at hand it seemed also possible that the intense light might have caused photochemical decomposition in the retina with the slow liberation into the blood stream of some depressor substance. To test this possibility a number of experiments were completed in which one eye was irradiated while the other was covered completely. After this both eyes were excised and separately treated in various ways. Some were ground up and extracted with Ringer solution, in other cases the retina alone was removed, ground and extracted, while in still other cases the retina was removed and allowed to lie in the extracting solution without grinding. Alcoholic extracts were also made. The extracted material was then injected, sometimes into the same animal, sometimes it was allowed to extract 24 hours and was then injected into another animal. The results of this entire series of experiments, 30 in number, was wholly inconclusive and more work must be done before any decision will be justified.

Microscopic examination of sections made from eyes exposed to such very intense light has failed to bring out any *histological* evidence of photochemical action of such a degree as to account for these results.

It should be emphasized that neither of the two sources of light employed here give any short ultra violet rays. In case of the lantern the rays passed through the glass bulb and through three glass lenses. While the carbon arc used was rich in ultra violet rays the condensing lens filtered out the short rays below 3300 Å rather sharply as shown by a spectogram.[3]

The eyes of dogs exposed to either light frequently showed interesting reactions. It will be recalled that an effort was made in all experiments to maintain a stage of anesthesia that permitted of a corneal reflex. Not infrequently, after a few minutes' irradiation, the eyeball would undergo oscillatory movements, in a few cases even rotatory movements were observed. In still other cases, retraction of the eyeball was noticed. This was seldom ever sustained longer than a few seconds.

It was rarely necessary to employ retraction of the eyelids. The pupil occasionally constricted to a very narrow opening and usually dilated again after some time, indicating fatigue of the constrictor mechanism. In most

[3] All spectograms were made by Mr. F. C. McDonald of the Ryerson Physical Laboratory.

cases, however, even when a strong corneal reflex was present, the pupil remained unchanged.

While irritation and consequent reflexes probably play some part in these responses to light, it was apparent that these factors alone could not account for the pronounced effects. With no probability of massive production of any pressor substance, there remains only the effect of the light on the blood as it flows through the retinal capillaries. Subsequent experiments undertaken with this point in mind will be reported in another paper.

Thanks are due Dr. A. J. Carlson and Dr. A. C. Ivy for suggestions and criticisms in the conduct of these experiments; also to Mr. McDonald and other members of the Department of Physics for free use of time and facilities; also to Dr. C. J. Herrick, Dr. Jeannette Obenchain and Dr. L. B. Arey for assistance in the preparation and examination of histological material.

SUMMARY

1. A beam of light of approximately 100 f.c. intensity, when directed into one or both eyes of a dog under ether anesthesia will cause a pronounced depression of arterial blood pressure.

2. A beam of light from a carbon arc employed in the same manner and giving a total intensity of 1250 f.c. will produce a more profound depression of blood pressure. More intense light does not produce a change of proportionate magnitude but induces the same degree of change somewhat more rapidly.

3. This effect is not wholly due to nervous factors since bilateral vagisection and section of the optic nerve do not abolish nor prevent the effect though the latter operation does modify considerably.

4. No positive evidence was obtained that photochemical decomposition liberated any depressor substance into the blood stream.

5. There remains, then, only the effect of light on the blood, to be considered as a mechanism of sufficient potency to produce these effects.

6. Short ultra-violet rays were not present in either of the two sources of light employed.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF LIGHT

III. Effects on Arterial Blood Pressure of Direct Irradiation of Blood in Vivo

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From the Hull Physiological Laboratory of the University of Chicago

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In the preceding paper of this series (Reed, 1925b), it was shown that irradiation of the eyes of dogs under ether anesthesia produced a pronounced fall in blood pressure; also, that this effect occurred when the optic nerve of the irradiated eye, or both vagi, were sectioned. It was further pointed out that there was no positive evidence of liberation into the blood stream of any depressor substance. These facts made it appear that the fall in blood pressure must be brought about as a direct effect on the blood circulating in the retinal capillaries. It is the purpose of this paper to present the results of experimental investigation of this possibility.

The thickness of the skin of dogs as well as the heavy deposits of pigment in the deeper layers made it a matter of some difficulty to find readily accessible in the dog another rich capillary bed. For this reason it was decided to make use of the mucosa of the mouth and pharynx. Dogs were anesthetized with ether by tracheotomy and the mouth spread wide open. A beam from a carbon arc of 1250 f.c. intensity was directed upon the exposed mucosa. The lamp was connected on a 110 v.D.C. circuit and operated at 4 amperes. Since a heavy glass condensing lens was employed there were no ultra violet rays present of wave lengths shorter than 3300 Angstrom units. (Fig. 3, *2*.) This procedure was employed on three dogs, resulting in average depression of arterial blood pressure of 60 per cent, maximum 79 per cent, minimum 21 per cent.

Since the mucosa was protected from drying and the surface temperature thereof did not increase to a degree detectable with an ordinary thermometer, it is apparent that such profound results could only have been produced by direct effect on the blood.

But in an effort to eliminate the possibility of other factors, the following technic was employed. A glass tube of 3 mm. bore was fitted with cannulas at each end and interposed in an artery, usually the carotid, although the femoral was employed in a few cases.

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Since coagulation time was greatly diminished under irradiation, a great deal of difficulty was encountered in the tendency to clotting in the tube. However, a series of 8 experiments was completed in which it was possible to maintain unimpaired circulation throughout the duration of the experiment. But in most experiments heparin was employed intravascularly before irradiation was begun (Reed, 1925a). The beam was focussed on the blood stream flowing through the tube. The adnexa were protected from irradiation and from drying by heavy pads of cotton soaked in Ringer solution.

In experiments in which heparin was not employed coagulation time was greatly diminished as compared to control experiments with ether anesthesia, by irradiation of the eye or of the blood directly by any portion of the spectrum of the carbon arc. This phase will be discussed in detail in a later paper.

The source of light was a special carbon lamp[2] operated at 4 amperes, 110 v.D.C., and fitted with a quartz lens. The condensed beam at 20 inches gave a photometric reading of 4000 f.c. intensity (*1*, fig. 4).

In series 10, (fig. 1, curve *10*) are included 8 experiments in which the blood was irradiated directly through a glass lens and a glass tube so that the spectrum below 3300 Å was cut off. At the end of one and one-half hours blood pressure had fallen to an average level of 32 per cent of the original level, a depression of 68 per cent. Heparin was not employed in this series.

In series 11 (fig. 1, curve *11*) the same procedure was employed except that each of the 6 animals received an optimum injection of heparin before irradiation. Depression of blood pressure, as indicated by the composite curve, did not begin so early nor proceed so rapidly and the ultimate depression was not as great, though still within the range of experimental variation. The average depression was only 45 per cent.

Series 12 (fig. 1, curve *12*) includes 7 experiments in which an effort was made to determine the possible effect of heat on the total reaction. Already it had been determined that rectal temperature was not affected and a thermometer placed alongside the arterial tube never registered above 30°C. In this series exactly the same technic was employed as in series 11 except that a screen[3] was interposed to filter out the infra red

[2] This was the "Spectro-sun" lamp, manufactured by the Paul E. Johnson Co., Chicago, Ill., and loaned for research purposes by Mr. Johnson. Certain carbon electrodes were supplied by the National Carbon Co. others by Dr. Axel Reyn of Copenhagen, Denmark.

[3] This consisted of a gold plated filter manufactured by the Acme Motion Picture Co. of Chicago, and loaned by Dr. I. S. Falk of the Ricketts Bacteriological Laboratories. It was capable of filtering out 95 per cent of heat rays without diminishing luminous intensity more than 10 per cent. Some short ultra violet rays were removed also.

rays. (Fig. 3, *2*.) A correction of the spectrogram shows that no rays were transmitted of greater wave length than 7000 Å. Recalling that the blood was heparinized it is apparent that in this case absence of heat rays resulted in greater depression of blood pressure. While this may be still an experimental variation it is, on the other hand, possible that the infra red rays may partially neutralize the effects of the short wave rays, though this is not in accord with the work of Hill and his co-workers (Leonard Hill, 1925).

In series 13 (fig. 1, curve *13*) were included 11 experiments in which the same spectrum employed in series 12 (fig. 3, *2*) was applied to one eye.

In series 15 are included experiments in which a beam of 4000 f.c. intensity at site of application was generated with tungsten core carbon sticks (N.C.C., fig. 4, *3*), giving a rich spectrum down to 2250 Å. A quartz tube with 2 mm. bore was placed in the artery. The blood was heparinized in every case to the extent of 10 mgm. per kilo of body weight. At the end of one hour and fifteen minutes of irradiation the average depression was 52 per cent, less than that for series 10 in which the short ultra violet rays were filtered out.

There were many experiments in each of these groups in which the degree of shock was so profound as to result in the death of the animal even after the anesthetic was discontinued very early. As an example of this experiment 156 was plotted as shown in figure 2. This experiment belonged in series 10, although there was another very similar to it in series 15. Blood pressure fell in 20 minutes of irradiation from 152 mm. Hg, which pressure had been sustained for 15 minutes prior to irradiation, to 48 mm. or 32 per cent of the initial level. At this point anesthesia was discontinued. During the period represented by the broken line in the graph, many small fluctuations occurred which were too complex to be plotted. The general level of pressure declined progressively although irradiation was continued only 25 minutes. The animal died in a condition of profound shock two hours and ten minutes after irradiation was begun. Post-mortem examination revealed no pathological condition which could have accounted for this extraordinary result. This experiment was so striking that Doctor Carlson and Doctor Ivy were called in to witness it and vouch for the actual occurrence. While this was an extreme example, there were many other animals that died in a condition of shock.

It appears then that direct irradiation of circulating blood will in some way, not yet determined, produce a profound condition of shock which may terminate fatally for the animals. That this is not due to direct heating of the blood is indicated by the fact that rectal temperature was not increased. Furthermore, in experiments in which the gold screen was used comparable results were obtained.

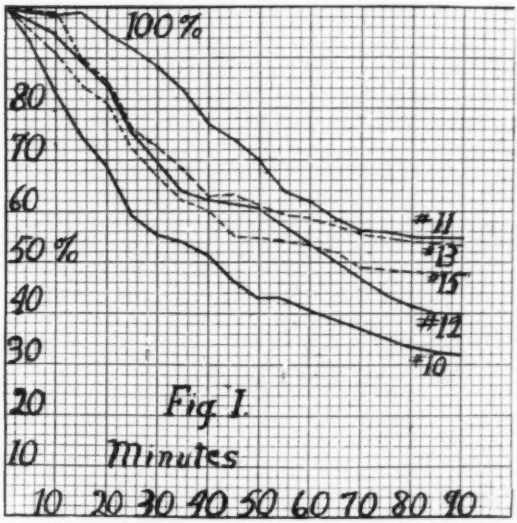

Fig. I.

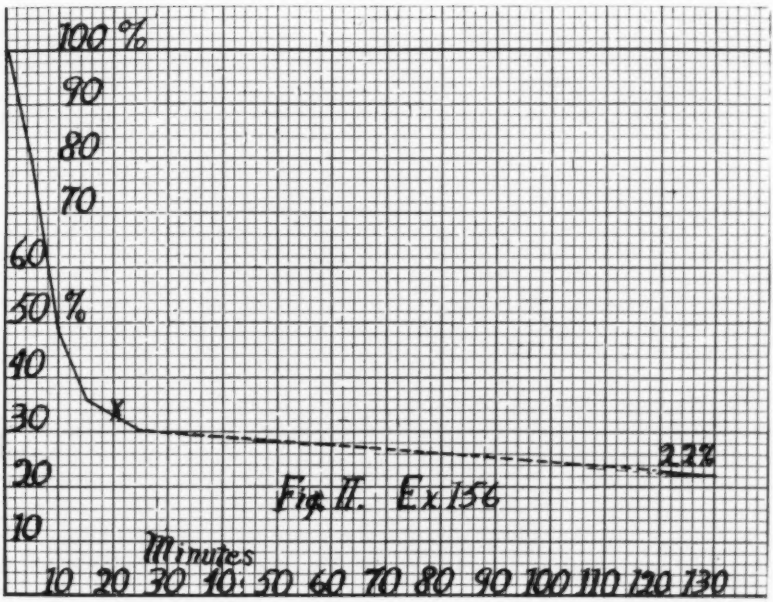

Fig. II. Ex 156

In this connection, however, it is well to call attention to the fact that Sonne (1921) has carried out a series of experiments which he interprets as showing that luminous rays have "the capacity of heating an essential portion of the aggregate blood volume of the organism to a temperature which may, according to all probability, exceed the highest fever temperature ever measured without causing body temperature to rise to any appreciable degree." It is known that blood exhibits a strong absorption

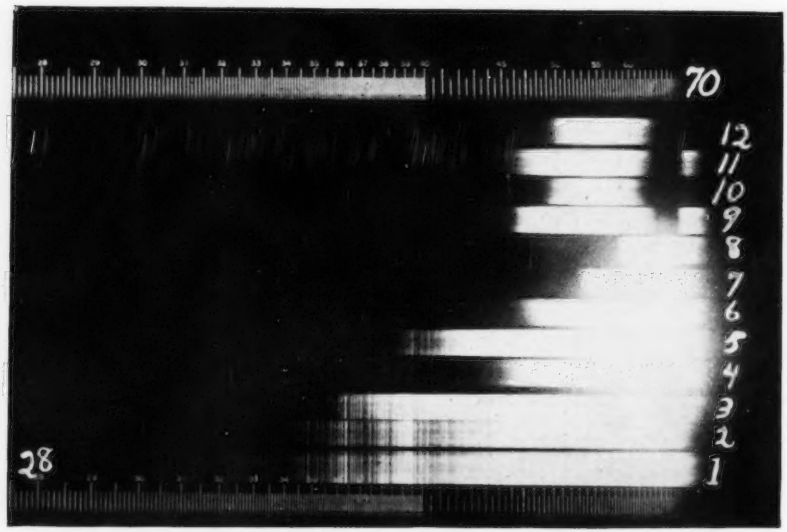

Fig. 3. Spectrograms obtained in one minute exposure of magnetite electrodes through quartz lens.

1, total spectrum; *2*, same through gold filter; *3*, same through viopake glass; *4*, same through Wratten kodak filter no. 4; *5*, same through Wratten kodak filter no. 2; *6*, same through Wratten kodak filter no. 9; *7*, same through Wratten kodak filter no. 16; *8*, same through Wratten kodak filter no. 23; *9*, same through Wratten kodak filter no. 40; *10*, same through Wratten kodak filter no. 58; *11*, same through Wratten kodak filter no. 60; *12*, same through Wratten kodak filter no. 61.

bond up to 4500 Å. According to Grotthus' law only light absorbed can bring about any chemical change. It is, therefore, conceivably possible that this portion of the spectrum, when absorbed, causes some specific change in the blood. At any rate, the result is certainly not due alone to the shorter ultra violet rays that have been heretofore assigned such a large place in the biology of light.

A few oncometer determinations on intestinal volume indicate that abdominal viscera are not greatly congested. Direct inspection showed

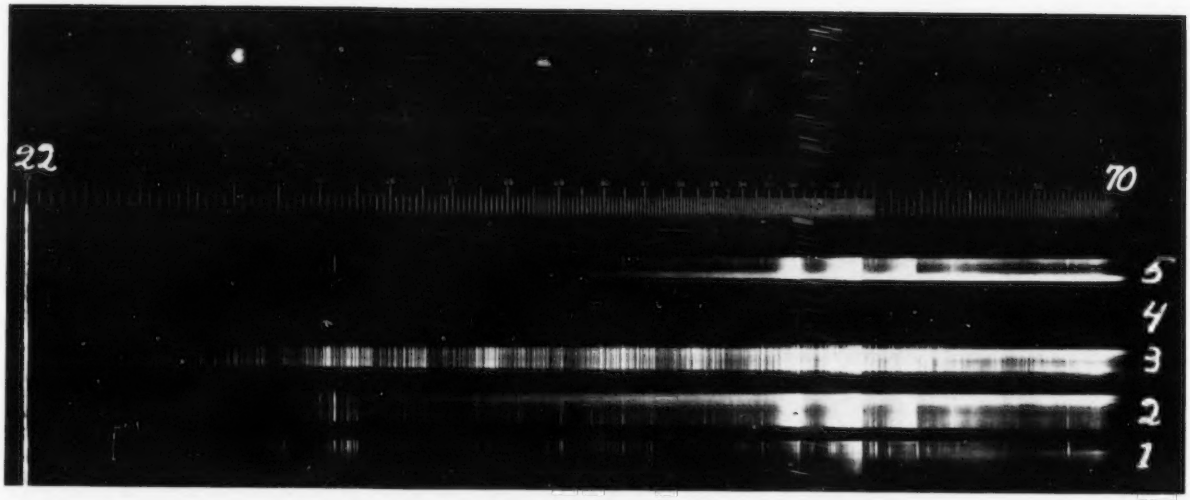

Fig. 4. Spectrograms obtained on one-minute exposure except no. 4.

1, naked carbon arc; *2*, large size tungsten core carbons (English); *3*, small size tungstens core carbons (N.C.C.) supplied by National Carbon Co.; *4*, same, 2 minutes' exposure through filter of para nitrosodimethyl aniline and 20 per cent copper sulphate; *5*, carbon electrodes supplied by courtesy of Dr. Axel Reyn, Finsen Light Institute, Copenhagen, Denmark.

the viscera to be only slightly more congested in appearance than when blood pressure is well maintained. On the other hand, the skin capillaries were very noticeably dilated and engorged.

It is not possible to state at present what is the mechanism by which this great shift of blood volume occurs. Evidently the explanation is to be found in some physical or chemical change in the blood itself. Studies along these lines are being carried on at present and such data as have been collected will be reported in a subsequent paper.

It is recognized that the conditions of irradiation in these experiments are very extreme. Nevertheless, in view of the great confusion of reported results on various phases of light biophysics, it seems wise to impose extreme conditions in a standardized experiment and determine what changes actually occur. These points having been established, it will then be possible to work back to less drastic exposures and determine modifications of results in accord with modified procedure.

Clinical results of photodynamics, at least in certain conditions, are no longer in doubt. It remains, however, to establish the physiological mechanisms by which these results are produced.

SUMMARY

1. Direct irradiation of blood circulating through a quartz tube interposed in an artery will cause a massive depression of general arterial blood pressure. Animals sometimes die from the shock even when anesthesia has been discontinued.

2. This result may be obtained in irradiation with a carbon arc rich in ultra violet or with a spectrum from which has been filtered infra red rays (longer than 7000 Å), or ultra violet rays below 3300 Å. The differences between results obtained with these three spectra are not pronounced.

3. Since irradiation in vivo decreases coagulation time, it was usually necessary to make use of heparin intravascularly in order to keep the radiation tube clean. Heparin modified the average result to some extent.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF LIGHT

IV. Effects on the Blood of Irradiation in Vivo

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In a previous paper (Reed, 1925b) were reported effects on blood pressure of irradiating the blood of etherized dogs circulating through a quartz tube interposed in the carotid artery, with a beam of light of 4000 f.c. intensity projected from the positive electrode of a carbon-tungsten arc.[2] The spectrograms may be found in the above mentioned paper (fig. 4, no. *1*). The entire technic was described therein. It is the purpose of this paper to report the results of investigation of the changes in the blood itself, resulting from such irradiation.

In undertaking such a systematic study, one naturally looked for changes in the formed elements.

Many early investigators reported increase in the number of red cells and in the hemoglobin content resulting from irradiation. (Graffenberger, 1893), (Marti, 1897), (Bering, 1907). On the other hand, Borissaw (1900) found no change in these elements in dogs and rabbits confined in the dark as compared to control groups maintained in the light. Hanssen (1916) claimed that after carbon arc irradiation of patients there was a 7 per cent increase in hemoglobin content and a corresponding increase in erythrocytes. Burchardi (1921) reported an increase in both factors up to the stage of strong pigmentation Riedel (1921) insisted that there is such an increase but admitted it to be transient. Baumann (1920) cited four other authors who held that any increased readings of either erythrocyte count or hemoglobin content are due to concentration of the blood. Gelera (1922) insisted that neither hemoglobin nor erythrocytes are affected by phototherapy.

O'Elsnitz (1913) and Rollier (1923) suggested that the disagreement in reported observations is due to actual variability of response. This idea

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[2] The lamp employed in most of these experiments was the "Ultra-Sun" lamp manufactured by the Paul E. Johnson Co. of Chicago and loaned for research purposes through courtesy of Mr. Johnson. Part of the carbon electrodes were supplied by the National Carbon Co. through Mr. Johnson, part by Dr. Axel Reyn of Finsen Light Institute, Copenhagen, Denmark.

is supported by the observations of Taylor (1919) on the effects of insolation on 38 human subjects, 65.8 per cent of whom showed an actual increase in red count, 21 per cent showed a slight decrease, and 13.2 per cent showed no change. Bleyer (1903) reported that short exposures to tropical sun increased hemoglobin temporarily, but that later this substance was actually decomposed.

The reports of Cluzet and Kofman (1924) and of Pagniez, Ravina and Solomon (1924) make it clear that the effects of irradiation in vitro and in vivo are very different. On this account no consideration will be given in this paper to any results of such procedure with drawn blood since this investigation is concerned with the general systemic results.

Aschenheim (1913) maintained that therapeutic insolation always decreased the number of red cells so that the decrease in the amount of plasma in the peripheral vessels is not a factor in altered count. Orum (1908) maintained that darkness or red light will decrease the red count and hemoglobin at first but after two months both are increased by concentration of the blood. Blackader (1912) reported that during the cold dark season in northern Canada hemoglobin increased rapidiy, though temperature may have been a factor here. However, Blessing (1897), physician to Nansen on a polar expedition, found no evidence of any significant change.

Gröber and Sempel (1919) examined mine horses that had been immured for many years and found no evidence of any blood effects due to darkness. Laurens and Sooy (1924), however, have recently grown rats under four conditions varying from darkness to sunlight with the result that at the end of six months there was a progressively higher red count in each group as the intensity of light increased.

Using the same method that was employed in this work, Naswitis (1922) irradiated the blood of rabbits with a quartz lamp with the result that there was an increase in the number of red cells in blood drawn from the ear vessels. Since Traugott (1920) has shown that there is not much shifting of concentration of red cells in the blood stream, this would seem to be a definite result. Asada (1923) irradiated the spleen directly and found a transient massive increase in the number of erythrocytes followed by a slightly higher count for several days but with a parallel *decrease* in hemoglobin content.

Cramer and Drew (1923) and Clark (1922b) have called attention to the similarity between the effects of moderate doses of x-rays and radium and strong doses of light, so that it does not seem irrelevant to mention the work of Minot and Spurling (1924), Morris (1905), and Schweitzer (1916), all of whom report no appreciable change in red cells and hemoglobin as a result of x-ray therapy. Prolonged and excessive repeated exposure may lead to anemia (Portis, 1915), (Larkin, 1921). But as

with light exposures there is a great deal of conflicting evidence, many workers reporting an increase of one or both factors (Brill and Zehner, 1912), (Bucky and Guggenheimer, 1922), (Heim, 1922), (Schoeder 1922), while others find a decrease of one or both factors (Aubertin and Beaujard, 1908), (Case, 1922), (Hauenstein, 1921), (Henri and Mayer, 1903). Rud (1924) found, on examination of radiologic workers, no change in hemoglobin and red cell count. Amundsen (1923) had previously reported similarly while Capparetti (1922) found a slight decrease in number of red cells by x-radiation.

Barkus and Balderry (1924) have claimed that radiation of any kind will concentrate the blood. If this is true, and the nature of their observations renders the finding very plausible, then a higher hemoglobin reading is easily accounted for.

Since Ohta (1924) has found that after injection of rabbits with hematoporphyrin, sunlight actually causes unquestionable decrease in red cell count, it is probable that a sufficiently sensitive animal would respond to insolation or phototherapy in such a manner.

In this study hemoglobin readings were taken with a Dare hemoglobinometer. The following tabulation of readings in a series of dogs under ether but without irradiation shows a tendency toward concentration of the blood even in so short a period as 90 minutes or even an hour, although in a few cases the final reading was lower than the first one.

	EXPERIMENTS													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Ave.
Hemoglobin, per cent A.....	98	88	70	95	85	105	100	95	88	97	105	98	90	93+
Hemoglobin, per cent B.....	95	96	70	90	80	110	110	100	99	110	110	110	98	98+

Increase 5 per cent.

In another series of animals in which the blood was irradiated directly, hemoglobin readings were of the order shown in the following tabulation:

	EXPERIMENTS						
	1	2	3	4	5	6	Ave.
Hemoglobin, per cent before irradiation.....	100	105	90	80	85	65	88
Hemoglobin, per cent after irradiation.....	100	110	90	98	100	78	98

Increase 10 per cent.

The difference between these two series is too small to be of significance and it must be acknowledged that no conclusions of an increase in hemoglobin under a period of irradiation such as employed here is justified. Nor would one expect this result to follow from such application.

Red cell counts showed an average of 7,000,000 cells per cubic millimeter in normal dogs. The count at the end of the experiments was usually somewhat higher but never beyond the limits of experimental error. It is possible then that some concentration of blood did occur since both groups gave similar results but it is evident that this short period of irradiation of blood directly did not produce any physiological increase in either hemoglobin or red cells.

Quite different, on the other hand, were the results of white cell counts. Krebs and Meyer (1903) observed that patients treated with hot air and incandescent light baths showed a leucocytosis. Bering (1907) found the same in mice irradiated with the Alpine sun lamp. O'Elsnitz (1913) stated that the total white count of patients or normal individuals was *usually* increased immediately but later decreased below the original level. Aschenheim (1913) found 80 per cent general leucocytosis after direct insolation, with both relative and absolute lymphocytosis. Large mononuclear leucocytes were as often decreased as increased. Polito (1908) confirmed this observation in rabbits exposed directly to sunlight. According to Staübli (1913) insolation at high altitude caused a leucopenia with relative lymphocytosis and mononucleaosis. Taylor (1919) stated that a massive dose of x-rays produces a lymphopenia while a mild dose produces a lymphocytosis. Baumann (1920) quoted a number of investigators, as follows: Stäubli found a decrease in total white count with relative lymphocytosis in phototherapy; Bordenheuer, Baer and Engelsmann, and Berner all confirmed this observation; while Waltschoff found in both man and rabbits a constantly increased total white count with relative neutrophilosis; Wernshied agreed on the first point but insisted on a relative lymphocytosis. Baumann, himself, was able to confirm Waltschoff.

Clark (1921) investigated extensively the effect of iron arc irradiation on rabbits. She reported that the region below 3000 Å. did not alter neutrophiles but produced a persistent lymphocytosis. The region from 3300 Å. to 3900 Å. depressed both lymphocytes and polymorphonuclears, the latter to a less degree. The visible region increased both types of cells while red and infra-red alone produced no effect beyond a transient lymphopenia. Murphy and Sturm (1919) have shown that dry heat alone will produce a pronounced lymphocytosis. Gelera (1923) found that ultra-violet light produced a great increase in polynuclear neutrophiles and large mononuclears and a temporary increase in small lymphocytes, the first exposure producing the greatest change. Eosinophiles and basophiles were not affected. Capparetti (1922) found among radiologists a progressive leucopenia with relative lymphocytosis. Penetti (1923) found the total white count increased in guinea pigs by diffuse light or by injection of eosin and leaving in the dark.

Ramain (1923) made an extensive study of patients irradiated in various

ways. Insolation produced lymphocytosis and leucocytosis. In 8 out of 30 patients there was leucopenia but all were normal in 48 hours. In 20 cases exposed to ultra-violet rays from a mercury vapor lamp sharp leucopenia occurred in 10 cases while the others showed leucocytosis with progressive fall to leucopenia. Infra-red rays produced leucopenia with quick recovery.

Amundsen (1923) found leucopenia with relative lymphocytosis in radiologists. Asada (1923) in irradiation of the spleen in rabbits found a variable reaction in white cells. Minot and Spurling (1924) found as a result of x-radiation, leucopenia and lymphopenia which was very persistent. Vedder (1924) found that oxidase granules in leucocytes disappear on irradiation with ordinary light only if the temperature is raised above 62°C. With ultra-violet light they disappear without heating. Mouquin (1924) maintains that after deep radiotherapy there may be leucopenia persistent for months, with lymphopenia. Den Hoed (1924) claims that irradiation is followed by high leucocytosis for a short period, then leucopenia; the rise in total number may not occur. Rud (1924) found the total white count low among radiologists. Spurling and Lawrence (1925) have found that radium irradiation of blood produced no change in the white cells.

While the figures accumulated in this study are too extensive to reproduce here in full, it was found that direct irradiation of the blood with a carbon arc invariably produced a massive decrease in the total white count with high *relative* lymphocytosis but absolute lymphopenia. Because there was no constancy of counts of large mononuclears, eosinophiles and basophiles and no consistent effects could be noted, these groups were disregarded and all cells classified as polymorphonuclears and lymphocytes. The following tabulation of control experiments will dispose of the anesthetic as a factor in producing this change. That it was not due to shifting of cells to another part of the circulation was shown by the fact that blood counts were made from samples drawn from various regions though routinely counts were made immediately from samples drawn from the femoral artery. Counts made in various regions were always comparable, both total and differential.

	TOTAL	POLYMORPHONUCLEAR	LYMPHOCYTES
Control experiments			
		per cent	*per cent*
(1) Early	8,209	68	32
(1) Late	9,000	71	29
(2) Early	24,000	72	28
(2) Late	25,900	70	30

		TOTAL	POLYMORPHONUCLEAR	LYMPHOCYTES
Irradiation experiments				
(1)	Before	20,100	63	37
	After	5,600	38	64
(2)	Before	13,400	71	29
	After	5,900	35	65

The results as tabulated are too pronounced to be due to any other factor than the irradiation. Traugott (1920) has shown that massive shifts of white cells do not occur without mechanical interference or inflammatory irritation. Most of these experiments were not over one and one-half hours' duration so that even without the numerous controls the results are definite. There was no direct correlation between the degree of depression of blood pressure and the hemoclastic effect. Nor was it found possible to differentiate the results obtained by irradiation with the various portions of the spectrum as indicated above. In most of these experiments and even in control animals, the blood was heparinized as previously described (Reed, 1925a). So far there has been found no evidence that heparin in any way influences any of the factors considered in this report.

In all there were 60 irradiation experiments in which blood counts were taken. Without exception the total white count was decreased, the extent of decrease varying from 40 per cent to 80 per cent. The shift in percentage was always in the direction typified by the above examples. In no case, however, was the inverse ratio sufficiently pronounced to give an absolute lymphocytosis.

As an illustration of the progressive nature of the change taking place, the following detailed protocol is given. The first count was made just prior to the beginning of irradiation, the other three at the indicated intervals after the procedure was begun:

	TOTAL	POLYMORPHONUCLEARS		LYMPHOCYTES	
		Per cent	Actual	Per cent	Actual
First count	21,000	56	11,800	44	9,200
20 minutes of irradiation	15,800	51	8,100	49	7,700
50 minutes of irradiation	10,000	36	3,600	64	6,400
60 minutes of irradiation	6,000	33	1,980	67	4,020

In this case the animal died in profound shock shortly after. This probably bore no direct relation to the degree of hemaclasia, however, since many other animals survived a longer period of irradiation with as great a reduction in total white count.

It was not possible to discover any mobilization of leucocytes or lymphocytes anywhere in the body, so it must, for the present at least, be assumed that they were destroyed by the irradiation. Since Viale (1919) has shown that substances which are biologically photodynamic are also lipoidolytic, their action must be intracellular. Furthermore, since it has been repeatedly shown that oxygen is necessary for any bio-photodynamic effect, it is fair to assume that the general effect is in the direction of oxidation. This assumption is partly borne out in the findings of a few investigators that irradiation of any kind speeds up metabolism. All of these facts then point to a penetration of the cells by rays of appropriate wave length with resultant activation of oxidative processes. If this acceleration exceeds certain limits there is destruction and disintegration of protoplasm with consequent injury to the organism as a whole. The resistance of red blood cells to the intense treatment given in these experiments is in keeping with their state of metabolism. On the other hand, destruction of white cells is in line with all the work which tends to show destruction of bacteria by raying. Even more specific is the work of Hinrichs (1924) on lower forms which underwent photolysis very quickly after irradiation. In the light of this explanation it is not difficult to understand the divergent results of various observers. It is apparent that at different stages of the photodynamic process one might easily obtain divergent results. Also it is apparent that there is a widely variable range of susceptibility in individuals.

It will, however, be necessary to repeat these observations in survival experiments before any final conclusions can be drawn. The status of blood cells *after* the period of irradiation will be of great interest and importance. Such experiments have been undertaken but have so far not been successfully completed. Further efforts will be made in the near future.

SUMMARY

1. A beam of light of approximately 4000 f.c. intensity projected from the positive electrode of a carbon-tungsten arc lamp operated on a 110 v. D.C. circuit at 4 amp. and condensed through a quartz lens was directed upon a quartz tube interposed in the carotid artery so that the blood flowing through the tube was irradiated directly

2. Minor changes in hemoglobin percentage and in erythrocyte count occurred in both irradiated and control dogs to about the same degree so that it is probable that the increase was due to concentration of the blood.

3. There was an absolute leucopenia, a lymphopenia but a relative lymphocytosis. The changes were not due to the anesthetic nor to the shifting of leucocytes.

4. All available evidence indicates that photodynamic effects are, biologically, stimulators of oxidation; and it is suggested that rays constitute one means of accelerating metabolism, which may, however, proceed to a degree of intensity that is destructive.

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IRRADIATION OF CHICKS WITH COD LIVER OIL[1]

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The efficacy of cod liver oil and ultraviolet light in the cure and prevention of rickets has been adequately established by numerous investigations.

Kugelmass and McQuarrie (1) have demonstrated the photo-activity of substances curative of rickets, and conclude: "These experiments point strongly to the common property of emitting ultraviolet rays, of cod liver oil, egg yolk, sperm oil, bile, hydroquinone on the one hand and of sunlight or quartz mercury vapor radiation on the other, as the basis for their identical curative action in rickets."

Steenbock and Black (2) have reported that when rats irradiated with ultraviolet light and non-irradiated rats were kept together in the same cage that "approximate equality of growth was obtained." They state further: "By irradiation with the quartz mercury vapor lamp, rat rations can be activated, making them growth promoting and bone calcifying to the same degree as when the rats are irradiated directly." Hess (3) reports similar results by irradiating vegetable oils and adds, "the potency of cod liver oil is also enhanced by irradiation."

The results obtained in the investigations cited above suggested to the writer the possibility that if cod liver oil contained some form of ultraviolet energy it could be shown by irradiating animals with it.

It is the purpose of this paper to report upon the results obtained by irradiating young chickens with cod liver oil.

Experimental. Two groups of newly hatched White Leghorn chicks were used as the test animals. All chicks were of similar breeding and from flocks under uniform conditions. Both groups were brooded in the laboratory in small pens $3\frac{1}{2} \times 3\frac{1}{2}$ feet in floor area. The floors of the pens were made of wood and wood shavings were used for litter. The heat was supplied by an electric brooder. No direct sunlight was available at any time.

The ration for each group was essentially the Wisconsin chick ration

[1] Published with the approval of the Director of the Agricultural Experiment Station, Purdue University, Lafayette.

(4) without the mineral ingredients and consisted of 80 grams ground yellow corn, 20 grams wheat middlings and buttermilk ad libitum. The omission of the minerals from the complete Wisconsin formula showed the ration employed to be highly rachitic for young chicks, as had been reported (5).

The ground corn and wheat middlings were thoroughly mixed and fed ad libitum in small wooden troughs. In the feed trough for group 2 there were placed two half-liter Pyrex flasks which were nearly filled with cod liver oil.

These were so arranged that, while feeding, the chicks were compelled to bring their bodies in contact with the flasks in order that they might become irradiated as much as possible. The flasks also were in contact with the feed, thereby permitting irradiation on the feed at all times.

Under the hover of the brooder, during the first three weeks, there were placed three eighth-liter Pyrex flasks and two small quartz tubes, so arranged that the chicks would come in direct contact with the vessels when under the hover. The space under the hover was restricted so that it was nearly completely filled by the chicks and the Pyrex flasks of cod liver oil. On the 18th day more space was given under the hover and three other eighth-liter Pyrex flasks were added. All vessels were left unstoppered to permit slow oxidation of the oil. The openings were covered by thin muslin and so protected that it was impossible for the chicks to imbibe any of the oil. The cod liver oil was changed twice weekly after the first two weeks to insure irradiation from highly potent oil. A highly antirachitic oil was used in the experiments.

Pyrex containers were used since this glass had been found to transmit considerable ultraviolet light (2), (6).

Results. All chicks in each lot developed rickets, but two males and four females in the irradiated group were still living when the experiment was discontinued at the end of seven weeks. These survivors would probably have lived from one to two weeks longer.

Table 1 shows the number of days survived by each chick and table 2 shows the maximum weight obtained by each chick. These data clearly show that some benefit was obtained from the irradiation with cod liver oil. The lack of severity of rickets and the postponement of rachitic symptoms were characteristic of the irradiated group.

Figures 1 and 2 show the growth curves of the two groups of chicks. It is interesting to note that for the irradiated chicks all curves have a decline (at point indicated by *A*) but an incline immediately follows, even in those which died a few days afterwards. It was at this point that three additional flasks were placed under the hover to provide more irradiation.

Discussion. According to the work of Kugelmass and McQuarrie (1)

it might be expected that under suitable conditions it would be possible to demonstrate biologically an emission of ultraviolet energy from cod

TABLE 1

Days survived

GROUP 1—IRRADIATED				GROUP 2—NON-IRRADIATED			
Females		Males		Females		Males	
Chick number	Days survived	Chick number	Days survived	Chick number	Days survived	Chick number	Days survived
1	28	2	42	37	27	40	26
5	34	3	25	38	29	44	33
6	42	4	49+	39	33	45	34
7	49+	8	49+	42	19		
9	49+	11	41				
10	36	13	40				
12	28						
14	49+						
15	49+						
Average..	40.4+		41.0+		27.0		31

Note: The plus (+) after chicks 4, 7, 8, 9, 14 and 15 indicates that these chicks were still living at the end of 49 days when the experiment was discontinued.

TABLE 2

Maximum weights attained by each individual

GROUP 1—IRRADIATED				GROUP 2—NON-IRRADIATED			
Females		Males		Females		Males	
Chick number	Maximum weight	Chick number	Maximum weight	Chick number	Maximum weight	Chick number	Maximum weight
	grams		*grams*		*grams*		*grams*
1	58	2	74	37	65	40	81
5	67	3	70	38	88	44	90
6	88	4	110	39	77	45	80
7	90	8	118	42	57		
9	122	11	91				
10	62	13	106				
12	57						
14	123						
15	80						
Average...	83.0		94.8		71.7		83.7

liver oil. While rickets was not entirely prevented its onset was sufficiently postponed to indicate a definite effect from irradiation with cod liver oil.

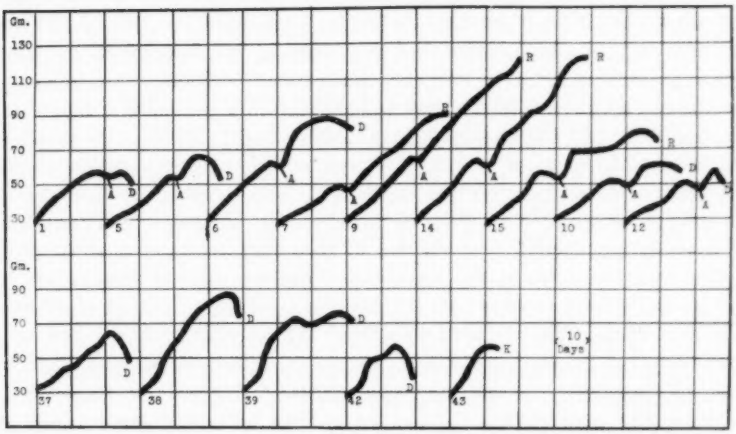

Fig. 1. This chart shows the growth curves of the female chicks in irradiated group (upper series) and non-irradiated group (lower series). *A* indicates the point at which three additional Pyrex flasks of cod liver oil were placed under the hover. *D* indicates dead, *K* indicates killed, and *R* indicates removed at the end of the experiment.

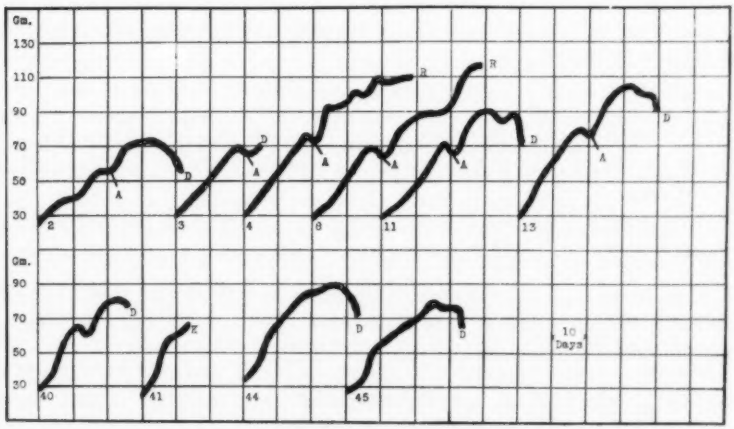

Fig. 2. This chart shows the growth curves of the male chicks in irradiated group (upper series) and non-irradiated group (lower series). *A* indicates the point at which three additional Pyrex flasks of cod liver oil were placed under the hover. *D* indicates dead, *K* indicates killed, and *R* indicates removed at the end of the experiment.

The results of this experiment would not indicate that the methods used would have practical application in prevention or cure of rickets, but serve only to demonstrate that the antirachitic substance in cod liver oil is some form of ultraviolet energy since it can be transmitted in a similar manner as ultraviolet light is transmitted and produces a biological response like that obtained from ultraviolet light. It is quite possible that a suitable calcium and phosphorus addition to the ration might have shown definite prevention of rickets, but a highly rachitic ration was purposely selected in order to secure a severe test. Furthermore, the marked susceptibility to rickets of the chick, compared with other species, would emphasize the certainty of the response to irradiation.

The results here reported are in harmony with the findings of Kugelmass and McQuarrie (1) who employed the photographic method and concluded that the antirachitic substance in cod liver oil is a form of ultraviolet energy.

During the preparation of this paper Hess and Weinstock (7) have reported that cholesterol and phytosterol, as well as other substances, "can be rendered antirachitic by radiations from the quartz mercury vapor lamp," and further state: "A series of tests with selective filters showed that the radiations which render cholesterol active biologically are similar in their wave lengths to those which have been found to protect animals against rickets when they are directly exposed to the rays." This would suggest very strongly that cod liver oil and other antirachitic substances might contain some form of ultraviolet energy as our experiments have indicated.

CONCLUSIONS

1. The antirachitic substance in cod liver oil can be transmitted externally to young chickens by means of irradiation with cod liver oil in Pyrex flasks.

2. The results obtained present further evidence that the antirachitic substance in cod liver oil is some form of ultraviolet energy.

I wish to acknowledge my indebtedness to Mr. L. P. Doyle of the Veterinary Department of this institution for his assistance in making the post-mortem examinations on the chicks.

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STUDIES ON THE ULTRAFILTRATION AND ELECTRODIALYSIS OF INSULIN SOLUTIONS

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The methods used thus far for the purification of insulin may be divided into two classes; fractional precipitation of the protein-like substances by regulation of the hydrogen ion concentration, and precipitation by various reagents. Among the first type may be noted the methods of Doisy, Somogyi and Shaffer (1923), (1924), and among the second, those of Collip (1924), Dudley (1923), Dudley and Starling (1924) Moloney and Findlay (1923), Best and Scott (1923), Shonle and Waldo (1924), and N. B. Fischer (1923). The only departure from either of the above methods is the meager and rather incomplete work of Locke and Hirsch (1924a), (1924b) on electrodialysis.

In this investigation the purpose was to find a method for separating or concentrating the active constituent in insulin solutions that would avoid the addition of precipitants, absorbants and buffers now commonly used. It was the aim of the authors to obtain the active material simply and quickly in sufficient purity for chemical investigation.

Successful application of ultrafiltration through graded membranes, and of methods of electrophoresis and electrodialysis to the separation of amyloses in starch were lately carried on in this laboratory (Taylor and Iddles. Dissertation, Columbia, 1925). These methods suggested themselves in attempting to purify insulin solutions.

Although the use of the first method did not give the desired result in the way of purification, nevertheless it did bring out some interesting properties of insulin. For this reason the technique has been given in some detail. On the other hand, the second method lent itself nicely to concentration of the physiologically active substance.

EXPERIMENTAL WORK. *A. The ultrafiltration of insulin solutions through collodion membranes of varying permeability.* The insulin[1] solu-

[1] The authors wish to express their thanks to Dr. G. H. A. Clowes, through whose courtesy the Eli Lilly Company supplied insulin for this investigation.

tions used were the clinical U-20 iletin (Insulin-Lilly) (1923). An apparatus, employing membranes, made and graded according to Nelson and Morgan (1923–24), served to give all the data herein recorded. The plates, on which the membranes were made, were floated on mercury to insure perfect leveling, the only departure from the Nelson and Morgan technique.

The ultrafiltration unit consisted of the parts, shown assembled and correspondingly lettered in figure 1.

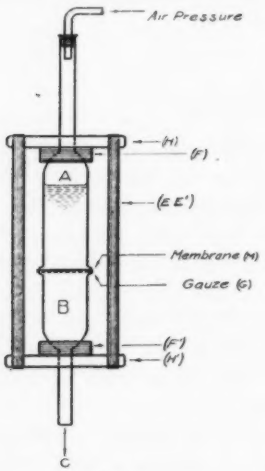

Fig. 1

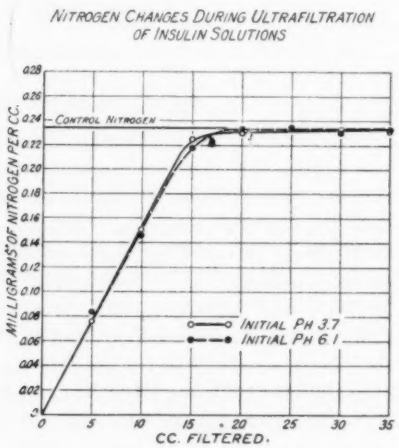

Fig. 2

Fig. 1. *A*, Gooch funnel serving as an insulin compartment; *B*, Gooch funnel serving as the lower half of the ultra-filter; *C*, a receiving vessel for the filtrate; *M*, a permeable membrane; *G*, a nichrome gauze to support the membrane and prevent its rupture during filtration; *EE'*, elastic bands to hold the upper and lower funnels rigid; *HH'*, wooden collars, fitted on the funnels, over which the bands *EE'* were stretched; *FF'* felt buffers to prevent fracture of the glass due to the pressure exerted by the bands, *EE'*.

A tight seal between the upper and lower funnels, and the membrane was effected by means of a rubber-paraffin cement.

When the apparatus had been tested and found to be leak-proof, an insulin solution, usually 15 cc., was placed in the upper part *A*, and a receiving vessel was placed in position *C*. Air, freed from carbon dioxide by passage through soda lime, and kept under a constant pressure of 60 cm. of water by means of a manostat, was then applied to the upper part of the apparatus and the preliminary filtration was begun. When the filtrate gave a distinct precipitate with phosphotungstic acid, the filtration was stopped.

The necessity for this preliminary filtration and test with phosphotungstic acid became apparent from some introductory experiments on ultrafiltration. These showed that before any conclusions could be drawn regarding the permeability of collodion membranes to insulin as measured by the physiological activity of the filtrates, it was essential first to saturate the membranes to be used with insulin. This was accomplished best by the preliminary filtration. The saturation of the membrane could be determined easily by the formation of a white precipitate when a solution of phosphotungstic acid (Sherman, 1912) was added to the filtrate. The absence of a phosphotungstic acid precipitate indicated that no active principle had passed into the filtrate, usually because the membrane had not become saturated. The proof that a filtrate giving no precipitate with phosphotungstic acid is physiologically inactive is shown by the following experiment:

Five cubic centimeters of clinical U-20 insulin were ultrafiltered through a grade 15.7 membrane, which had not been saturated previously by a preliminary filtration. The filtrate gave no precipitate with phosphotungstic acid while the control sample gave a copious one under the same treatment. Samples of filtrate and control were tested biologically for blood sugar reducing properties.

The physiological activity in these samples and in all samples mentioned in subsequent work was tested out on rabbits weighing about 2 kilos. The standard injection per animal per kilo of body weight was one physiological insulin unit, which has been defined by the Toronto Insulin Committee (1923) as, "one-third of the amount required to lower the blood sugar below 0.045 per cent and cause convulsions in a rabbit weighing 2 kilograms, which has been previously starved for 24 hours, within 2 to 5 hours after injection." Three samples of blood were drawn from each animal, an initial sample, one $\frac{3}{4}$ hour after injection, and one $1\frac{3}{4}$ hours after injection. The sugar in each sample was quantitatively determined by the Shaffer and Hartmann (1921) method.

The results of these physiological tests on the filtrate and control sample are given in table 1.

From the above data, it can be seen readily that the filtrate which gave no precipitate with phosphotungstic acid, was totally inactive, while the control, which gave a copious precipitate was active.

I. Effect of ultrafiltration on physiological activity. In all subsequent experiments, the membranes were saturated with insulin by a preliminary filtration, and then washed three times with 10 cc. of distilled water. A fresh insulin solution then was placed in part *A*, a clean receiving vessel at *C*, and the regular filtration was begun.

When about one-half of the insulin solution had passed through the through the membrane, the filtration was stopped. Samples of control,

TABLE 1

GRADE OF MEMBRANE USED	DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER ¾ HOUR IM MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A – B) BLOOD SUGAR DROP AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A – C) BLOOD SUGAR DROP AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
15.7	Control	135	74	80	61	55	Clinical U-20 insulin gave good precipitate with phosphotungstic acid
15.7	Control	141	66	68	75	73	
15.7	Control	130	68	74	62	56	
Average		135	69	74	66	61	
15.7	Filtrate	110	108	110	2	0	Gave no precipitate with phosphotungstic acid
15.7	Filtrate	122	118	118	4	4	
15.7	Filtrate	124	128	128	0	0	
15.7	Filtrate	122	128	122	0	0	
15.7	Filtrate	108	128	128	0	0	
15.7	Filtrate	105	118	114	0	0	
Average		115	121	120	0	0	

filtrate and remainder on the membrane, were tested for physiological activity as described above.

This experiment was repeated for several membranes of varying grades of permeability with the hope of obtaining some fractionation of the active from the inactive material, as would be evidenced by increased activity of the solution above or below the membrane. Table 2 gives the results obtained.

From the above data, which represent averages of series of animal results on each sample tested, it is seen that within the experimental error, the average sugar drop per unit injected is the same for the control, filtrate and remaining solution. It may be concluded, then, that the active principle of insulin preparations can be ultrafiltered through collodion membranes of varying permeability without loss of physiological activity, and further, that no apparent fractionation takes place, during the ultrafiltration.

II. Nitrogen changes during ultrafiltration of insulin solutions. The practicability of ultrafiltration of insulin solutions through graded membranes without loss of physiological activity having been shown, it was decided next to study the nitrogen content of the filtrate, with the view of ascertaining whether nitrogen could be removed from insulin by ultrafiltration without impairing its activity. This could be accomplished best, by collecting fractions of the filtrate and determining the nitrogen content and physiological activity in each.

The ultrafiltration apparatus and membrane technique used was the same as described in the previous section, with the exception that each successive five cubic centimeters of filtrate were collected in separate vessels, and the membrane was not saturated previously. The nitrogen content in each fraction was then quantitatively determined by the micro-Kjeldahl technique of Pregl (1923), and each fraction was tested for precipitation with phosphotungstic acid. This was done prior to testing the fraction for physiological activity, because as has been mentioned previously, the absence of a phosphotungstic acid precipitate indicates the absence of activity in the filtrate.

Several fractional ultrafiltration experiments were carried out with membranes of different degrees of permeability ranging from 2.0 to 12.0, (graded according to Nelson and Morgan (1923–24)) and with insulin solutions on both the acid and alkaline sides of pH = 4.8 (the recorded isoelectric point of insulin (Council on Pharmacy and Chemistry 1923)). The results obtained in all these investigations are shown by the detailed data from two experiments, both on the same grade membranes, but on different sides of the isoelectric point. Concurrently, a colorimetric determination of the hydrogen ion (Clark, 1924) concentration on each fraction was made. The data obtained have been recorded in table 3 and are shown graphically in figures 2 and 3.

TABLE 2

GRADE OF MEMBRANE USED	DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A − B) BLOOD SUGAR DROP AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
20.7	Control	137	61	61	76	76	Clinical U-20 insulin 1 unit per kilo was injected
12.0	Control	122	63	73	59	49	
11.5	Control	135	69	71	66	64	
Average		131	64	68	67	63	
20.7	Filtrate	127	75	59	52	68	Filtrate from U-20 insulin through membranes whose grade is given in first column
19.6	Filtrate	124	69	65	55	59	
12.0	Filtrate	121	68	64	53	57	
12.0	Filtrate	120	66	56	54	64	
11.5	Filtrate	134	64	62	70	72	
3.6	Filtrate	134	71	53	63	81	
Average		127	69	60	58	67	
20.7	Remainder	110	64	57	46	53	Solution on membrane when filtration was stopped
19.6	Remainder	136	74	62	62	74	
12.0	Remainder	115	52	41	63	74	
11.5	Remainder	123	59	56	64	67	
3.6	Remainder	128	69	52	59	76	
Average		122	63	53	59	69	

Figures are averages for:
3 rabbits on samples: Control 20.7, 12.0, 11.5. Filtrate 20.7. Remainder 20.7.
4 rabbits on samples: Filtrate 3.6. Remainder 3.6.
5 rabbits on samples: Filtrate 12.0.
6 rabbits on samples: Filtrate 19.6, 12.0, 11.5. Remainder 19.6, 12.0, 11.5.

From the above results it is apparent that a low nitrogen content of the filtrate, probably caused by loss in saturating the membrane, is accompanied by an equivalent drop in physiological activity.

Since collodion membranes removed hydrogen ion from solutions (Bethe and Toropoff, 1914, 1915), it is to be expected, that the insulin solution above the membrane should become progressively more alkaline as the ultrafiltration continues. As soon as a pH of about 4.8 was reached, precipitation of insulin started. On the alkaline side of the isoelectric point, this change in hydrogen ion did not take place and the precipitation of insulin on the membrane was avoided.

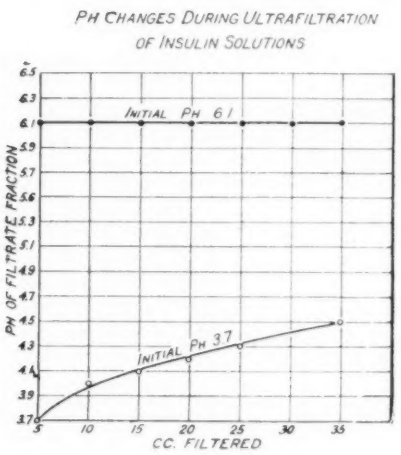

Fig. 3

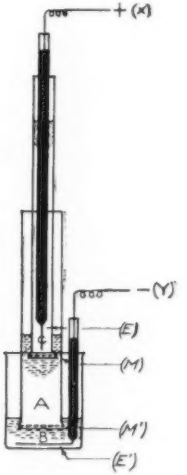

Fig. 4

Fig. 4. *A*, an insulin compartment filled with about 25 cc. of U-20 insulin (Lilly); *BC*, electrode compartments filled with 20 cc. of distilled water; *EE'*, the platinum electrodes; *MM'*, impermeable membranes.

B. THE ELECTRODIALYSIS OF INSULIN SOLUTIONS. *I. The formation of an active sediment by electrodialysis of insulin solutions.* The inactivation of insulin by both oxidation and reduction (Shonle and Waldo, 1923), demanded the use of a special type of electrolytic cell for the investigation of the behavior of the active principle when subjected to electrodialysis. The essential feature in the design of the cell must be, therefore, complete removal of the electrodes from the insulin solution undergoing electrodialysis. This would help prevent oxidation or reduction of the active principle by any secondary products of the electrolysis, formed at the electrodes.

The electrolytic cell: The cell finally adopted and used in all experi-

TABLE 3

PART A

Grade membrane used = 10.0.
Initial pH = 3.7 (acid side of isoelectric point).

DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC.	B BLOOD SUGAR AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC.	C BLOOD SUGAR AFTER $1\frac{3}{4}$ HOURS IN MILLIGRAMS PER 100 CC.	D; (A − B) BLOOD SUGAR DROP AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC.	E; (A − C) BLOOD SUGAR DROP AFTER $1\frac{3}{4}$ HOURS IN MILLIGRAMS PER 100 CC.	NITROGEN CONTENT IN MILLIGRAMS N_2 PER CUBIC CENTIMETER	pH OF FILTRATE FRACTION	REMARKS
Control	117	52	65	65	52	0.234	3.7	Clinical U-20 insulin
Filtrate fraction no. 1						0.076	3.7	No precipitate with phosphotungstic acid
Filtrate fraction no. 2						0.151	4.0	Very slight precipitate with phosphotungstic acid
Filtrate fraction no. 3						0.225	4.1	Some precipitate with phosphotungstic acid
Filtrate fraction no. 4						0.230	4.2	
Filtrate fraction no. 5						0.233	4.3	Heavy precipitate with phosphotungstic acid
Filtrate fraction no. 6	143	81	70	67	73	0.233		Volume too small for pH
Filtrate fraction no. 7						0.231	4.5	

PART B

Initial pH = 6.1 (alkaline side of isoelectric point).

DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC.	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC.	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC.	D; (A − B) BLOOD SUGAR DROP AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC.	E; (A − C) BLOOD SUGAR DROP AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC.	NITROGEN CONTENT IN MILLIGRAMS N_2 PER CUBIC CENTIMETER	pH OF FILTRATE FRACTION	REMARKS
Control	126	86	67	40	59	0.234	6.1	U-20 insulin adjusted to pH 6.1
Filtrate fraction no. 1						0.084	6.1	No precipitate with phosphotungstic acid
Filtrate fraction no. 2						0.146	6.1	Very slight precipitate with phosphotungstic acid
Filtrate fraction no. 3						0.218	6.1	Precipitate with phosphotungstic acid
Filtrate fraction no. 4						0.233	6.1	
Fraction no. 5	122	64	62	58	60	0.235	6.1	Heavy precipitate with phosphotungstic acid
Filtrate fraction no. 6						0.231	6.1	
Filtrate fraction no. 7	135	60	49	75	86	0.233	6.1	2 rabbits took convulsions

ments on electrodialysis, was composed of the parts shown assembled and correspondingly lettered in figure 4.

The insulin compartment, A, and electrode compartment, C, were sealed off by means of collodion membranes, MM', made in such a manner as to render them impermeable to the active principle but permeable to electrolytes during the electrodialysis.

The electrodialysis membranes: The membranes, MM' mentioned above, were prepared by the following method. The end of the cell unit upon which the membrane was to be made, was covered with a tightly stretched piece of cheese-cloth, (shown as dotted lines in fig. 4) which served as a framework for the membrane. The covered end of the unit was then dipped into a fairly pure collodion solution (Merk's collodion), removed, and revolved for ten minutes. At the end of this time all of the ether and most of the alcohol had evaporated, leaving on the cloth gauze a rather impermeable membrane. This was repeated three times, thereby insuring against leaks, and giving a very impermeable membrane. These membranes then were soaked in distilled water for at least five hours before being used. They did not "blush" in contact with water but remained perfectly transparent.

The electrodialysis technique: When the cell had been set up as shown in figure 4, the insulin solution[2] to be electrodialysed was placed in compartment A, the proper volume (20 cc.) of distilled water in compartments B and C, and the current turned on. A potential of 100 volts was applied between x and y by means of direct current from the lighting service, passing through suitable resistance. With this drop in potential across x and y, a current of 5 milliamperes flowed through the cell at the outset. In all subsequent experiments the polarity of the cell, unless otherwise stated, was that shown in figure 4, with C the anode and B the cathode compartment.

Current was passed for any desired period of time. The circuit was then broken, compartment A was disengaged from the cell, and the liquid decanted from the sediment which had been formed on the membrane M during the electrodialysis.

The sediment was washed into a small Büchner funnel with absolute methyl alcohol. The residue on the filter was washed several times with absolute alcohol and finally with absolute ether. This last treatment is not absolutely essential, but it produces an almost white sediment instead of a dark brown one and materially increases the ease with which the substance can be removed from the paper. The sediment was removed from the filter and dried in vacuo at 30°C. to constant weight.

[2] More concentrated solutions of insulin were diluted until they were equivalent to U20 (Lilly). This contains approximately 2 mgm. solids per cubic centimeter of which from the present work about 1 mgm. appears to be physiologically active.

Since insulin is reported to become inactivated when heated above 30°C. (Best and Macleod, 1923; Doisy, Somogyi and Shaffer 1923; Dudley 1924; Murlin, Piper and Allen, 1923) none of the samples was heated higher than that temperature. All subsequent data are reported on constant weight at 30°C. in vacuo.

II. The physiological activity of the sediment. The physiological activity of the sediment produced by the electrodialysis of insulin solutions was tested, in order to determine the effect of the electric current on the potency of the active principle.

The dried sediment was weighed out in 2 to 2.5 mgm. samples, and dissolved in such a volume of physiological salt solution (0.9 per cent sodium chloride solution) acidified with 4 drops of N/10 hydrochloric acid per 2 cc. of solution, as would give a concentration of 1 mgm. per cubic centimeter. Then $\frac{1}{2}$ cc. of this solution was diluted to 10 cc. with physiological salt solution, giving a final concentration of 0.05 mgm. per cubic centimeter of solution. The standard injection was 1 cc. of this diluted solution or 0.05 mgm. per kilo of body weight. Control samples were made up in exactly the same manner to contain about 0.1 mgm. per cubic centimeter of final solution, and were injected at about 0.1 mgm. per kilo of body weight. The animal technique and blood sugar determinations were carried out as described under ultrafiltration. Several samples of sediment prepared from different batches of insulin, together with adequate controls, were tested for physiological activity as described, and the results obtained have been recorded in table 4.

From the data given in table 4, it can be seen that the average blood sugar drop for $\frac{3}{4}$ hour and $1\frac{3}{4}$ hours after injection, on 7 control samples, was 61 and 67, while the average on 10 comparable sediment samples for the same periods of time, was 66 and 68 mgm. per 100 cc. of blood. The average activities were about equal. From table 4 it also will be noted that the unit weight of solid material injected per kilo was not equal for the control and sediment samples, the latter being only 0.05 mgm. as compared to 0.1 mgm. of the former, or one-half as much. Since half the unit weight of sediment produces about the same lowering of blood sugar in the experimental animals, as a whole unit weight of original insulin, it has been concluded that the sediment produced by the electrodialysis of insulin solutions is apparently twice as active, physiologically, as the original material. As will be shown later, these results take into consideration the experimental errors.

III. The physiological activity of the filtrate. The greater activity of the sediment could be explained best by assuming that some relatively inactive substance was being removed from the active material during the electrolysis of insulin solutions, or in other words, the original material was being fractionated in some way into two substances, one of high activ-

TABLE 4

EXPERIMENT NUMBER	DESCRIPTION OF MATERIAL	MILLIGRAMS PER KILO INJECTED	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A − B) BLOOD SUGAR DROP ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
1 and 2	Control	0.12	120	58	59	62	61	Clinical U-20 iletin (Insulin-Lilly)
3	Control	0.10	129	81	63	48	66	
4 and 5	Control	0.13	124	65	60	59	64	
7 and 8	Control	0.10	134	74	57	60	77	
9 and 10	Control	0.12	129	77	77	52	52	
15	Control	0.10	135	67	58	68	77	
12, 13, 16	Control	0.10	144	67	71	77	73	Concentrated non-clinical iletin. diluted to U-20 concentrated
Average			131	70	65	61	67	
1	Sediment	0.07	132	120	119	12	13	Insulin in contact with electrodes
3	Sediment	0.05	125	57	61	68	64	First active sample
4	Sediment	0.05	127	56	42	71	85	
5	Sediment	0.05	150	110	96	40	54	Precipitated from (CH_3OH—HCl) by dry ether
7	Sediment	0.05	142	74	63	68	79	Formed at pH 3.6—
8	Sediment	0.05	121	60	63	61	58	Formed in ½ hour at pH 3.6–3.7.
9	Sediment	0.05	135	74	69	61	66	From clinical U-20 iletin
10	Sediment	0.05	144	58	77	86	67	
15	Sediment	0.05	133	79	68	54	65	From clinical U-20 iletin
12	Sediment	0.05	127	72	77	55	50	From concentrated non-clinical
13	Sediment	0.05	127	70	69	57	58	iletin
16	Sediment	0.05	149	71	63	78	86	
Averages on nos. 3, 4, 7, 8, 9, 10, 15, 12, 13, 16			133	67	65	66	68	

Note: Figures above are averages on data from 3 to 4 rabbits per sample tested.

ity, and one of low activity. The validity of this assumption could be shown by the isolation of a relatively inactive product from the filtrate remaining after the removal of the active sediment.

This was attempted, and an organic substance showing little physiological activity was obtained from the filtrate by two different procedures. These consisted of *a*, evaporation of the clear filtrate from the electrodialysis to constant weight at 30°C. in vacuo; and *b*, adjustment of the pH to 4.8, filtration of the precipitate formed and drying the latter in vacuo as usual. Method *a* gave a substance which showed greater activity than the material produced by *b* as can be seen from the physiological results on filtrate samples recorded in table 5.

The average drop of sugar per 100 cc of blood for the six filtrates was 28 and 15, as compared to 66 and 68 mgm. for the precipitates (table 4) for the same time intervals after injection, thereby showing that the physiological activity of the organic substance remaining in the filtrate after removal of the active material is less than half that of the sediment. Even though these figures do not represent an absolute cleavage of the original insulin into an active and inactive substance, which would be a very difficult accomplishment as shown by some of the work presented in following sections, nevertheless they do indicate that the production by electrodialysis of a substance of increased activity is due to fractionation of the original material.

IV. The deposition of the active sediment. The deposition of an active sediment during the electrodialysis of insulin solutions had been established, so far, but no change either of the polarity of the cell or of the initial pH of the solution had been attempted. It was of interest to investigate both of these changes from the point of view of determining whether the active material always deposited at the same electrode, or migrated in different directions, depending on the hydrogen ion concentration of the solution.

The usual electrodialysis was carried out with the polarity of the cell arranged so that the lower membrane was the cathode, and with the pH of the solution about 3.7, the acid side of the isoelectric point. As has been stated, under these conditions, the sediment was always deposited at the lower or cathodic membrane. Under such experimental conditions, the cathodic deposition of the active material left two points open for discussion, namely, was the deposition on the cathodic membrane due to distinct cathode migration, or was it due to the material being drawn down to the lower membrane, which happened to be the cathode, merely by the force of gravitation.

In order to settle these points, experiments were performed with the initial pH of the solution at 3.7, but with the cell polarity reversed, thus making the lower membrane the anode, and the upper one the cathode.

TABLE 5

EXPERIMENT NUMBER	DESCRIPTION OF MATERIAL	MILLIGRAM PER KILO INJECTED	A. INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER 1¼ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A − B) BLOOD SUGAR DROP ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP 1¼ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
3	Filtrate	0.05	111	87	100	27	11	Produced by evaporation to constant weight at 30°C.
4	Filtrate	0.05	125	93	104	32	21	
15	Filtrate	0.05	127	82	111	45	16	
16	Filtrate	0.05	135	103	104	32	31	
12	Filtrate	0.05	139	129	132	10	7	Produced by adjustment of pH
16	Filtrate	0.05	126	103	122	23	4	
Average			127	99	112	28	15	

Under these conditions, the active material always migrated to the upper membrane against the force of gravity showing that when insulin solutions on the acid side of the isoelectric point were subjected to electrodialysis the cathodic deposition of active sediment was not due to gravitation, but was caused by a distinct cathodic migration of material carrying the activity.

The next point to be investigated was, obviously, the effect of changing the pH of the insulin solution from the acid to the alkaline side of the isoelectric point on the direction of migration of the active material. Experiments were performed with the pH of the insulin solutions adjusted to about 9.0 and kept on the alkaline side of the isoelectric point (pH = 4.8) by the continuous addition of dilute caustic soda solution. In all the experiments the deposition took place at the anodic, instead of at the cathodic membrane. Thus it has been shown that the deposition of an active sediment during electrodialysis of insulin solutions is caused by a definite migration of a material carrying the activity, and that the direction of migration is dependent upon the hydrogen ion concentration of the solution undergoing electrodialysis.

V. Deposition of the active sediment at a definite pH. In the preliminary experiments on the electrodialysis of insulin solutions, it had been observed that the pH of the solution changed as the electrodialysis progressed, as was to be expected, since the process combined dialysis with electrolysis. It was decided to study these pH changes more thoroughly with the idea of gaining perhaps more insight into the nature of the process which was causing the formation of the active sediment.

It has been shown in the previous section that the deposition of a sediment during electrodialysis of insulin solutions occurs on both the acid and alkaline side of the isoelectric point (pH = 4.8). The experimental difficulties involved in maintaining an alkaline solution during the electrodialysis, and more important, the rapid inactivation of insulin in alkaline solutions (Dudley, 1923; Shonle and Waldo, 1924) made it impossible to study the pH changes on the alkaline side of the isoelectric point. Therefore, only the pH changes on the acid side have been investigated.

The technique employed for the study of the pH changes during electrodialysis was of a type which not only followed every change in pH, but also determined the constancy of pH in different parts of the solution. The twofold result was accomplished by means of two capillary pipettes, one placed at the very bottom, and the other at the very top of the solution in the cell. By using a "Y" tube connection between the two capillary pipettes, it was possible to draw off liquid simultaneously from the very top and very bottom of the solution in the cell, thus minimizing the possibility of a change in pH by diffusion during the removal

of the samples. The pH of the top and bottom samples and also of the mixed solution was determined at different time intervals over a period of 24 hours by the colorimetric procedure previously referred to. The results obtained in three experiments on clinical U-20 insulin have been averaged, and are recorded in part A of table 6.

From these results it can be seen that when an insulin solution with an initial pH of 3.7 is subjected to electrodialysis there is a distinct drop of pH during the first part of the process, followed by a progressive increase in pH, and further, that these pH changes are the same in all parts of the cell within the experimental error of the pH determinations. It is

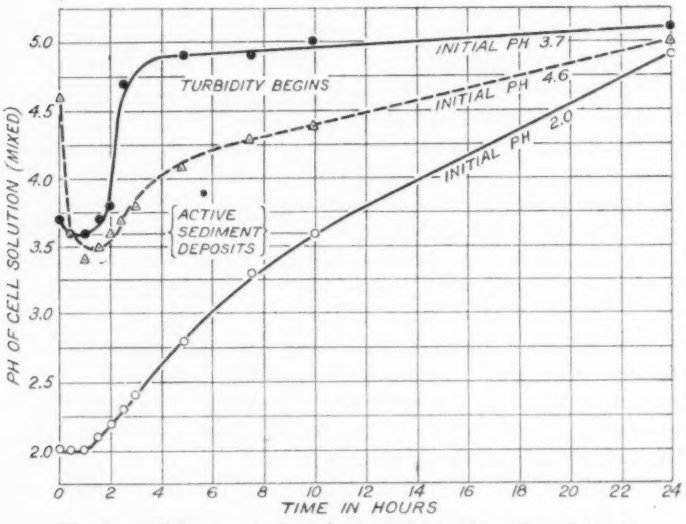

Fig. 5. pH changes during electrodialysis of insulin solutions

to be noted also, that accompanying the drop in pH of the solution is the deposition of all the weighable amount of active sediment. Furthermore, turbidity, followed by the formation of a boundary which very gradually settles down to the lower membrane, begins at a pH of about 4.7 to 4.8, or within the isoelectric range of the original insulin solution.

In order to determine whether these particular pH changes were specific for insulin solutions with an initial pH of 3.7, experiments were performed on solutions having a starting pH other than 3.7. Since the experiments recorded in part A of table 6 showed that the pH was constant throughout the entire cell, only the pH of the mixed solution was determined in the work which is about to be described.

TABLE 6

TIME	APPEARANCE OF SOLUTION	WEIGHT OF SEDIMENT DEPOSITED	DETERMINED pH OF SOLUTION		
			At top of cell	On mixed solution	At bottom of cell
Part A					
Start	Clear		3.7	3.7	3.7
½ hour	Clear	8.3 mgm.	3.6	3.6	3.6
1 hour	Clear	Not weighable	3.6	3.6	3.6
1½ hours	Clear		3.7	3.7	3.7
2 hours	Clear		3.9	3.8	3.8
2½ hours	Turbid		4.7	4.7	4.7
3 hours	Turbid				
5 hours	Turbid		4.9	4.9	4.9
7½ hours	Turbid		4.9	4.9	4.9
10 hours	Turbid		5.0	5.0	5.0
24 hours	Clear		5.1	5.1	5.1
Part B					
Start	Clear			2.0	
½ hour	Clear			2.0	
1 hour	Clear			2.0	
1½ hours	Clear			2.1	
2 hours	Clear			2.3	
2½ hours	Clear			2.3	
3 hours	Clear			2.4	
5 hours	Clear			2.8	
7½ hours	Clear			3.3	
10 hours	Clear	7.3 mgm.		3.6	
24 hours	Turbid			4.9	
Part C					
Start	Clear			4.6	
½ hour	Clear	7.2 mgm.		3.6	
1 hour	Clear	Trace		3.4	
1½ hours	Clear			3.5	
2 hours	Clear			3.6	
2½ hours	Clear			3.7	
3 hours	Clear			3.8	
5 hours	Clear			4.1	
7½ hours	Clear			4.3	
10 hours	Clear			4.4	
24 hours	Turbid			4.9-5.0	

Two experiments on clinical U-20 insulin with an initial pH of 2.0 and two on the same material but with an initial pH of 4.6, were performed, and the results obtained were averaged and recorded in table 6

parts B and C respectively. The data given in table 6 have been shown graphically in figure 5.

When the initial pH of the insulin solution was 2.0 nothing visible occurred during the course of the electrolysis until the pH had become adjusted to about 3.6, at which point practically the entire amount of active sediment deposited on the cathodic membrane, as was the case when the initial pH was 3.7. As the process continued, the pH of the solution gradually increased until, at a pH of about 4.8 the solution became turbid, as had been observed in the previous experiments.

When clinical U-20 insulin adjusted to an initial pH of 4.6 was electrodialysed, a very pronounced change in pH occurred during the first part of the process. The pH of the solution dropped from 4.6 to about 3.4 and all the weighable sediment was deposited on the cathodic membrane at a pH of about 3.6. As the electrodialysis continued, the pH of the solution progressively increased, until at a pH of about 4.8, the usual turbidity set in.

These results have led to the conclusion that the deposition of an active sediment during electrodialysis of insulin solutions having an initial pH on the acid side of the isoelectric point (4.8), always takes place at a definite pH, which appears to be about 3.6. It has also been concluded that the appearance in the solution of a turbidity at a pH of about 4.8, followed by the formation of a boundary which gradually settles to the lower membrane is due to an effect other than that which causes the deposition of the active sediment.

VI. Deposition of the active sediment is not due to isoelectric point precipitation. In the investigation of the pH changes in insulin solutions undergoing electrodialysis, it has been shown that the deposition of the active sediment takes place from a perfectly clear solution, when the pH has become adjusted to about 3.6. Furthermore, the clear solution becomes turbid at a pH of about 4.8. Since the isoelectric point of the original material is at a pH of about 4.8 (Doisy, Somogyi and Shaffer, (1923), (Council on Pharmacy and Chemistry 1923), and since the pH at which the deposition of the active sediment takes place is about 3.6, it appears, that the separation of the active material during electrodialysis of insulin solutions is not due to an isoelectric point precipitation.

The change in direction of migration of the sediment, as shown in section four, indicates that this material must be amphoteric. Until it can be demonstrated experimentally that the isoelectric point of the active sediment is at a pH other than 3.6, the argument advanced above is open to discussion because, if the isoelectric point is at 3.6 then the deposition of active sediment might be due to insolubility of the material at this particular pH. Several experiments were performed which prove that this deposition of active material was not due to isoelectric point precipitation.

In the first place, the isoelectric point of the active sediment was experimentally determined. Samples of dried sediment were dissolved in distilled water acidified with dilute hydrochloric acid. Very dilute alkali (N/200) was then gradually added with constant shaking until a precipitate appeared. The pH at which maximum precipitation occurred was determined colorimetrically and the addition of alkali was then continued until the precipitate dissolved, showing the amphoteric nature of the substance. Other samples of the same sediment were dissolved in distilled water, made alkaline with dilute alkali. The same procedure, as just described, was carried out excepting that dilute hydrochloric acid (N/100) was added instead of dilute alkali. The pH, at which the maximum precipitation occurred, or the isoelectric point of the active sediment, was found, as above described, to be about 5.2 Since the pH at which deposition of this material takes place has been shown experimentally to be about 3.6, while the isoelectric point of the sediment has been found to be at a pH of about 5.2, it can be seen that the deposition of active sediment during electrodialysis of insulin solutions is not due to an isoelectric point precipitation.

Other experimental proof for the validity of the above statement is had in the fact that if the circuit was broken just after the material had been deposited at a pH of about 3.6, the sediment entirely redissolved in a short time. This was to be expected, since this same active sediment is completely soluble in water at a pH of about 3.6.

Furthermore, if the insulin solution undergoing electrodialysis was agitated rapidly by stirring, no deposition of active sediment occurred even after two hours. When the stirring was stopped, the usual deposit formed. If an isoelectric point precipitation were the cause of the deposition of an active sediment during electrodialysis of insulin solutions, agitation of the solution should have no effect on the formation of the precipitate.

Therefore, it has been demonstrated by three independent experiments that the deposition of the active sediment from insulin solutions subjected to electrodialysis is not due to an isoelectric point precipitation.

The results of these experiments also show the difficulty of obtaining a filtrate with absolute physiological inactivity, due not only to the solubility of the active material at a pH of 3.6, but also to the overlapping of the isoelectric ranges (4.8 to 5.0 and 5.0 to 5.2) of the inactive and active constituents respectively.

The turbidity which has been shown to begin at a pH of about 4.8 and which is followed by the formation of a definite boundary and settling of the latter on the lower membrane, is probably due to isoelectric point precipitation of the inactive component. [This pH (4.8 to 5.0) is the isoelectric point of insulin usually recorded in the literature (Doisy, Somogyi and Shaffer, 1923; Somogyi, Doisy and Shaffer, 1924; Dudley,

1923; Council on Pharmacy and Chemistry, 1923).] This was shown experimentally by the failure of the flocculated material to redissolve when the circuit was broken, and to almost immediate resolution upon the addition of either acid or alkali.

The relative inactivity of the material flocculated out of the solution of a pH of about 4.8, due probably to isoelectric point precipitation has been shown by a quantitative experiment. A solution of clinical U-20 insulin with an initial pH of 3.7, was divided into two equal volumes. Both portions were independently electrodialysed in the usual manner. The process was stopped and the sediment removed from one solution when the pH had become adjusted to about 3.6, and from the second at a pH of about 4.8. The solids from each were dried as usual and tested for physiological activity on the same weight basis. The results obtained are recorded in table 7.

From these results it can be seen that the material flocculated at a pH of about 4.8 showed an average blood sugar drop of only 47 and 42, as compared to 68 and 79 for the sediment deposited at pH 3.6. This experimentally indicated that the material which settles out of the solution at a pH of about 4.8, after the sediment has become deposited at a pH of about 3.6, contains little physiological activity. Contamination of the active sediment by this product materially decreases the potency of the active substance. This experimental evidence strongly indicates a fractionation of insulin by electrodialysis, as suggested in section three, and shows also that it is essential to stop the electrodialysis of insulin solutions before the pH has become adjusted to about 4.8, in order to prevent contamination and subsequent decrease in potency of the active material by the relatively inactive substance produced at this pH.

VII. Chemical investigation of the active sediment. In the previous sections it has been shown that an active sediment is formed during the electrodialysis of insulin solutions, from causes other than isoelectric precipitation. An investigation into the physical and chemical characteristics of the active substance has been undertaken with the hope of throwing some light on the chemical nature of the active principle.

The active deposit from insulin solutions subjected to electrodialysis is white when first prepared, but on exposure to the air, it gradually develops a grayish color. Under microscopic examination the active sediment shows sharp, ragged edges, like pieces of broken glass but no definite crystalline structure.

The active substance is soluble in dilute hydrochloric, sulfuric, and acetic acids, and in dilute caustic soda solutions. It is insoluble in absolute and 95 per cent ethyl alcohol, absolute ether, acetone, carbon tetrachloride, benzene, petroleum ether, and ethyl acetate. It shows slight solubility in absolute methyl alcohol saturated with dry hydrogen chloride.

TABLE 7

DESCRIPTION OF MATERIAL	MILLIGRAMS PER KILO INJECTED	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER $\frac{3}{4}$ HOUR IN MILLIGRAM PER 100 CC. BLOOD	C BLOOD SUGAR AFTER $1\frac{3}{4}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D BLOOD SUGAR DROP AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP AFTER $1\frac{3}{4}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
Sediment	0.05	128	80	82	48	46	Formed at pH 4.8
Sediment	0.05	116	77	84	39	32	
Sediment	0.05	119	66	70	53	49	
Average		121	74	79	47	42	
Sediment	0.05	158	102	108	56	50	Formed at pH 3.6
Sediment	0.05	138	46	18	92	120	
Sediment	0.05	154	88	72	66	82	
Sediment	0.05	119	60	56	59	63	
Average		142	74	63	68	79	

The sediment when dissolved in acidified water, imparts a pale yellow color to the solution. In alkaline solution this yellow color is much more pronounced.

The active material is precipitated from acidified aqueous solutions by phosphotungstic acid. It can also be precipitated from aqueous solutions by the addition of a large volume of absolute ethyl or methyl alcohol. If dry ether is added to a solution of the active substance in absolute methyl alcohol saturated with dry hydrogen chloride, a non-crystalline product is precipitated which shows slightly less than normal physiological activity as can be seen from experiment 5 recorded in table 4. The average sugar drop for the material precipitated as described was 40 and 54, as compared to 59 and 64, in the same time intervals for the control sample.

The material burns readily, giving rise to an odor resembling burning hair and leaves a white fluffy ash. Quantitative determinations for the percentage ash in several samples of sediment have been recorded in table 8. The ash determinations were made in a small platinum dish on samples ranging from 20 to 30 mgm. by ignition at red heat to constant weight. The ash is not alkaline, and its insolubility in dilute acids prompted a chemical investigation. This was done by the usual method (Scott, 1922; Treadwell and Hall, 1915) with sulfuric acid and hydrofluoric acid, used in determining silica. The results obtained have been recorded in table 8.

It can be seen from the tabulated data, that the ash from three different samples of sediment consisted entirely of silica, while the ash from the control, from which these sediments had been produced, consisted of only 60 per cent silica. Since the ash from the control samples showed a distinctly alkaline reaction toward phenolphthalein, which the ash from the sediments did not, it can be seen that the electrodialysis has removed an alkaline constituent which is not essential to the physiological activity of insulin preparations. Lack of material made it impossible to determine the ash content of the other samples of sediment recorded in table 8.

Attempts to remove the silica from the sediments were made by means of ultrafiltration through impermeable membranes and by re-electrodialysis with reversed polarity, thus drawing the sediment against gravity but these were not successful. The percent of silica in the samples before and after these treatments was practically the same. The relatively low ash in sediment no. 16, with no decrease in physiological activity, indicates that the silica is probably not a part of the molecule of the active principle in insulin preparations.

A test for nitrogen, sulfur, and phosphorus by means of sodium fusions on active sediments nos. 12, 13 and 16 was positive for the first two elements. No positive test for sulfur, phosphorus, or halogens could be obtained on the filtrate samples nos. 12 and 16 (pH 4.8 material).

TABLE 8

SAMPLE NUMBER	DESCRIPTION OF MATERIAL	PER CENT ASH NON-VOLATILE AFTER IGNITION	PER CENT ASH NON-VOLATILE AFTER $H_2SO_4 - H_2F_2$ TREATMENT	PER CENT SiO_2 IN ASH	PER CENT N_2 BASED ON TOTAL SOLIDS	PER CENT N_2 BASED ON ORGANIC SOLIDS	REMARKS
4	Control				14.15		Clinical U-20 iletin (Insulin-Lilly)
9	Control				13.89		
10	Control				14.21		
15	Control				13.51		
12, 13, 16	Control	3.82	1.53	60.00	12.35	12.84	Concentrated non-clinical iletin
4	Sediment				18.68		Made from clinical U-20 iletin (Insulin-Lilly)
9	Sediment				13.88		
10	Sediment				13.66		
15	Sediment				13.62		
12	Sediment	2.71	0.00	100.00	14.62	15.02	Made from concentrated non-clinical iletin
13	Sediment	2.50	0.00	100.00	14.12	14.48	
16	Sediment	1.30	0.00	100.00	13.87	14.06	
Average						14.52	
4	Filtrate				12.18		From U-20 clinical iletin
12	Filtrate				13.78		From concentrated non-clinical insulin
13	Filtrate				13.40		
16	Filtrate				12.11		

Each figure represents the average of 2 determinations.

The nitrogen content of samples of sediment and filtrate was quantitatively determined by the micro-Kjeldahl technique of Pregl (1923). A sample weighing between 11 and 12 mgm. was used for each analysis. The weighed sample was dissolved in that volume of N/10 sulfuric acid which would give a final concentration of 1 mgm. per cubic centimeter. Five cubic centimeters of this solution, measured by a calibrated pipette, were used for each determination. This procedure made it possible to get 2 analyses on each sample using 5 mgm. of material for each determination. The results obtained from several analyses on samples of control, sediment and filtrate have been given in table 8.

From these data it can be seen that the percentage of Kjeldahl nitrogen in the sediments analyzed based even on total solids, is fairly constant, while the nitrogen content for sediments nos. 12, 13, and 16 based on total organic solids is constant within 0.96 per cent, and averages 14.52 per cent for the three samples.

The amphoteric nature of the active sediment, as shown by its ability to migrate toward either the anode or cathode, depending on the pH of the solution, and also by its solubility in both dilute acids and alkalies, when coupled with its precipitation by phosphotungstic acid, and its average nitrogen content of 14.52 per cent makes it appear that the active principle of insulin either is a protein-like substance or is very closely linked to some protein. It is certain that the active material which deposits during the electrodialysis of insulin solutions, shows characteristic protein reactions, even though the active constituent in this sediment may not itself be a protein.

VIII. A micro-crystalline compound from the active sediment. The appearance of the active sediment formed from insulin solutions subjected to electrodialysis, when examined under a microscope, indicated possibilities for crystallization. The relative insolubility of the active material in all general organic solvents rendered the usual crystallization technique inapplicable. Advantage was taken finally of the solubility of the active sediment in acidified solutions.

Samples of active sediment were dissolved in dilute aqueous hydrochloric, acetic, and sulfuric acid solutions. These were permitted to evaporate slowly with the hope of obtaining the sediment in a crystalline condition. Several attempts were made but each time the material precipitated from the solution in an amorphous condition.

Another series of attempts was made using dilute sulfuric acid and ethyl alcohol as the solvent for the active sediment. Some of the solutions produced a micro-crystalline compound in very small quantity after standing for several days. The optimum condition for crystallization has not been established definitely, but equal volumes of 25 per cent sulfuric acid and 95 per cent ethyl alcohol produced crystals from two

batches of active sediment in about 24 hours. This was the shortest crystallization time ever obtained, the usual time being at least one week.

The formation of the crystalline compound from the active sediment occurs in a very peculiar manner. The sediment, when first dissolved, gives a clear, pale, yellow solution. The micro-crystals, in very small quantity, form in this acid solution after some of the alcohol has spontaneously evaporated. On longer standing, the crystals disappear, giving way to a turbid solution, from which an amorphous material precipitates. As more solvent evaporates this amorphous substance redissolves, giving a brownish colored solution from which, sometimes, on very long standing, the micro-crystalline substance is again formed. In several attempts, it has been impossible to get back the crystalline product even after standing for weeks. If a little ethyl alcohol is added to the liquid containing the amorphous substance after resolution of the crystals, the former re-dissolves, and in some cases, re-crystallization has been accomplished in this manner. However, if a little water is added to the liquid containing the amorphous substance, no resolution occurs, but even more amorphous material seems to come out of solution. If a large volume of water is added, complete resolution occurs but no crystals have ever been obtained from such diluted solutions.

The crystalline compound produced as described, has always had the same form, and has always shown the same relative insolubility in ordinary organic solvents and in water. The insolubility of the crystalline material in water, and the very small quantities in which it has been produced, together with the great difficulty experienced in attempting to free it from traces of mother liquor, have made the physiological and chemical investigation of the compound very difficult.

From the two microphotographs, figures 6 and 7, some idea may be had of the general appearance of the crystals under the microscope. Figure 6 shows a typical batch of crystals in contact with the mother liquor, while figure 7 shows a single group crystallizing in the usual radiating manner about some dust spot as a nucleus.

These crystals which are inactive are formed at the expense of the active sediment by the treatment described above. Their crystalline form suggests tyrosine. This aspect of the work opens up a line of attack on the insulin problem.

Discussion of errors. The greatest error in this investigation was involved in the physiological assay of the activity in the various samples of material. This biological error is not due to faulty technique or inaccurate methods, but is due to the different reactions of the experimental animals to the insulin injection. In order to establish the validity of the conclusions drawn from the physiological tests, a series of at least

three animals was run on each sample. The average error, calculated from the mean deviation for each series was found to be ± 16.19 per cent for the blood sugar drop $\frac{3}{4}$ hour after injection, and ± 18.98 per cent for the blood sugar drop $1\frac{3}{4}$ hours after injection. The error is larger for the second interval after injection because the results at this point are influenced to a greater degree by the individual reactions of the experimental animals. The error involved in weighing out the 2 mgm. samples for the physiological tests becomes negligible in view of the much larger animal error.

The lack of material necessitated the use of colorimetric procedure for the hydrogen ion determinations. Since in this investigation, the colori-

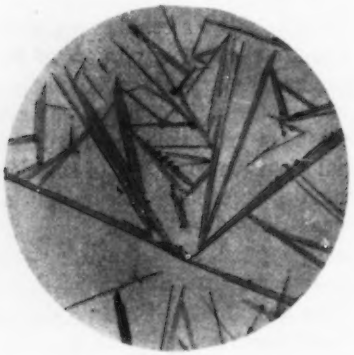

Fig. 6. Magnification 60×.
Ordinary light.

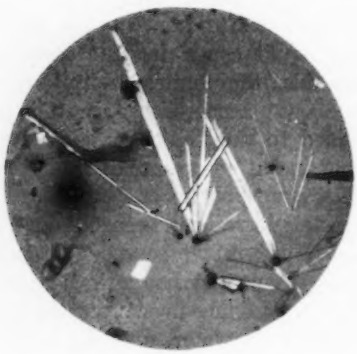

Fig. 7. Magnification 100×.
Green light.

Note: The authors wish to thank Mr. E. P. Polushkin of the Department of Metallurgy for these micrographs.

metric technique was open, not only to the usual optical and personal errors, but also to the so-called "protein" error, no greater accuracy than a close approximation is claimed for the hydrogen ion determinations. The results are, however, sufficiently accurate to indicate the general trend of the hydrogen ion changes in the cases investigated.

The largest error involved in the ash determinations is in the weighing of the samples (20 to 30 mgm.), and amounts to ± 0.33 to 0.50 per cent.

The micro-Kjeldahl technique was perfected by analyses on chemically pure samples of acetanilide. The accuracy of the method can be seen from the following typical analyses.

MATERIAL ANALYZED	WEIGHT OF SAMPLE	PER CENT N_2 FOUND BY ANALYSIS	PER CENT N_2 CALCULATED FROM FORMULA	PER CENT N_2 DIFFERENCE FROM THEORY
	mgm.			
c.p. acetanilid	6.6	10.53	10.37	+0.16
	7.4	10.70	10.37	+0.33
	7.4	10.82	10.37	+0.45
	7.2	10.82	10.37	+0.45
	7.1	10.70	10.37	+0.33

The standard sample weight in the actual analyses was 11 to 12 mgm. bringing into the method a possible error of ± 0.83 to 0.91 per cent.

All quantitative apparatus was calibrated and the errors involved were negligible in comparison to the errors discussed above.

SUMMARY

The results from A, ultrafiltration, and from B, electrodialysis have led to the conclusions summarized under the respective headings.

A. Ultrafiltration: 1. The active principle of insulin can be ultrafiltered through collodion membranes of high and low permeability without loss of activity.

2. The nitrogen content of insulin is not changed by ultrafiltration through collodion membranes of high or low permeability.

B. Electrodialysis: 1. An active sediment can be deposited from insulin solutions when subjected to electrodialysis.

2. The sediment produced by the electrodialysis of insulin solutions appears to be 100 per cent more active per unit of weight injected than the original material.

3. The filtrate, containing principally organic material, after removal of the active sediment, has little activity.

4. The electrode, at which the active sediment is deposited, is determined by the hydrogen ion concentration of the insulin solution undergoing electrodialysis.

5. There appears to be a definite hydrogen ion concentration at which the active sediment is produced from insulin solutions during electrodialysis.

6. The formation of the active sediment during the electrodialysis of insulin solutions, is not due to an isoelectric point precipitation.

7. The active sediment produced by the electrodialysis of insulin solutions shows protein reactions and contains organic sulphur but no phosphorus.

8. The inactive material shows protein reactions and contains neither sulphur nor phosphorus.

9. A micro-crystalline compound can be obtained from the active sediment formed by the electrodialysis of insulin solutions by treatment with an alcohol, sulfuric acid and water mixture.

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CHANGES IN THE PROTOPLASM OF NEREIS EGGS INDUCED BY β-RADIATION

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In the course of his investigations of the effect of radiation upon nereis eggs, Redfield observed that when a radiated egg was centrifuged it appeared to show a sharper differentiation between the resulting zones in the cytoplasm than was found in the unradiated egg. This suggested to him a decrease in viscosity. The possible theoretical significance of such a change led him to suggest to us the investigation of this point. We therefore undertook to repeat his experiment and, if possible, to make quantitative measurements of viscosity which might be correlated with the intensity and duration of radiation, in the same manner as Redfield and Bright, (1918), (1921), (1924) and Redfield, Bright and Wertheimer (1924) correlated the membrane-formation with radiation. It is of interest in this connection that in the case of certain plant cells Weber (1923) found no change of viscosity in the cytoplasm on radiation with x-rays. We were fortunate in the opportunity to collaborate with Doctor Redfield to the extent of utilizing the arrangements he had made for obtaining the material at Woods Hole and the radium emanation supplied him by the Harvard Cancer Commission.

METHOD. We hoped to develop a quantitative method of judging viscosity change by measuring the dimensions of the zones in the cytoplasm, resulting from centrifuging. A fertilized nereis egg when centrifuged shows three distinct zones (see fig. 1). At the top of the egg are concentrated the oil drops which are the lightest constituent of the cytoplasm. Beneath these is a hyaline zone which appears almost perfectly transparent. The lower half or two-thirds of the cell is occupied by the granular zone in which the heavier constituents of the cytoplasm are more or less densely packed. The cell in figure 1 is a good example of zone-formation in an unradiated egg, for the zones show well in profile. As Heilbrunn (1921) has pointed out, the less the viscosity of the cell, the more rapidly will the granular zone settle to the bottom when centrifuged. Therefore the percentage of the cell occupied by the granular zone should provide a rough measure by which to gauge differences in viscosity.

The difficulty in finding enough eggs so placed in the microscopic field as to enable us to observe the zones exactly in profile, and thereby make accurate quantitative measurements, rendered this procedure somewhat unsatisfactory, and prevented any such quantitative correlation as membrane-formation affords. We found, however, definite qualitative changes which could easily be photographed.

We sought to work with both fertilized and unfertilized eggs, but we found that, because of the great viscosity of unfertilized eggs (6), even the greatest centrifuging applied (9000 g. for 3 minutes)[1] failed to produce in them satisfactory zone-formation, the maximum effect being a concentration of oil drops and the beginning of a hyaline zone beneath them. We therefore devoted our principal attention to the study of fertilized eggs.

Fig. 1. Typical fertilized egg after centrifuging, showing zones in profile. Centrifuged with 8985 g. for 24 seconds. 20 minutes after fertilization. Photographed 50 minutes after fertilization. August 19, 1919. Magnification in all figures about 150.

Heilbrunn (1921) has reported a series of changes in viscosity observed by the centrifuge method in the eggs of nereis and other marine fauna, from the time of fertilization to the first cleavage. He finds in nereis a great decrease in viscosity soon after fertilization, and then transient increases in viscosity occurring at 20, 35, 47 and 65 minutes after fertilization, corresponding apparently with the prophases of maturation divisions and of cleavage mitosis and with the conclusion of the first cleavage. In order to obviate confusion due to these changes, we regularly fertilized the radiated and control eggs at the same time and centrifuged them together. We also varied the interval between fertilization and centrifuging in different experiments, so as to cover practically the entire period from 7 to 65 minutes after fertilization. We also made a number of control experiments in which unradiated eggs were centrifuged at various intervals after fertilization, for the express purpose of determining how much variation in our results was likely to appear in consequence of the natural fluctuations of viscosity which Heilbrunn has described.[2]

Our procedure in the earlier experiments with radiation was to divide the eggs from a freshly opened worm in two lots "R" and "C." Both lots were placed in sea water in watch glasses. Lot "R" was radiated, while lot "C" was kept in the same room at a safe distance from the radium emanation. The tube containing the emanation was placed horizontally over the eggs at a measured distance from them, varying from 3 mm. to

[1] g. is the centrifugal force measured in gravities.

[2] At the time of our experiments Heilbrunn's results were not yet published, but his experiments were in progress.

10 mm., and the intensity of radiation was calculated by the formula of Wood and Prime (1915) and estimated in millicurie-centimeters (Redfield and Bright, 1918). Radiation was continued for from 4 to 135 minutes. At a time varying from 2 minutes to 4 hours after cessation of radiation the eggs were fertilized. In the observations of Redfield and Bright (1918) the interval from radiation to fertilization made no difference in the membrane-formation, and presumably this interval was of no great significance in our experiments.

At first an electric centrifuge was used, and this was so constructed that four tubes of eggs could be centrifuged at once. After the first six experiments a hand centrifuge (Bausch and Lomb with hematocrit attachment) was installed in the room in which we worked, and thereafter was used in a large majority of experiments. This only accommodated two tubes at a time, but it had the following advantages: the speed, and therefore the centrifugal force, could be varied at will over a wide range, including a greater force than could be obtained with the electric machine; in this way the desired zone-formation could be induced more quickly.

In most of the experiments with fertilized eggs we mixed the radiated and control eggs before fertilization. All possible confusion from thus mixing the eggs was obviated by the fact that after fertilization radiated eggs are conveniently labelled by the swelling of the vitelline membrane, which has been the object of the studies of Redfield and Bright (1918). In the controls this membrane is visible close to the cytoplasm, whereas in the radiated eggs it is conspicuously enlarged. This procedure improved the experiments in several ways. The fertilization of radiated and control eggs was perfectly synchronized. Any slight difference inc entrifugal force which might result from a small difference in the lengths of the two tubes was eliminated. More important,—the radiated and control eggs could be studied and compared side by side in the same microscopic field. Finally, the two tubes of the centrifuge would accommodate four lots of eggs, and thus the results at two different times after fertilization could be studied in a single experiment; without mixing radiated and control eggs, this could only be done by using the four tubes of the electric centrifuge.

In estimating the centrifugal force, the formula cited by Heilbrunn (1921) was used in the case of the hand centrifuge. This varied in different experiments from 5000 to 11,400 times gravity. The duration varied from 9 seconds to 12 minutes. With the electric centrifuge we did not determine the actual velocity, but judging from the time required to induce zone-formation, the centrifugal force was considerably less than the maximum obtained by hand.

At first we endeavored to appraise our results by measuring with a

micrometer ocular the granular zone in as many eggs as could be found with the zone boundary showing in profile, and comparing this with the diameter of the whole egg. But since the granular zone is the heaviest, the eggs tend to settle with this zone at the bottom, consequently only a minority of zones could be seen in profile. The method proving unsatisfactory for quantitative results, we devoted our attention to noting qualitative changes in the cytoplasm and to making microphotographs to illustrate them.

Results. Although the results obtained with unfertilized eggs were meager, in three experiments there appeared to be slightly more of the hyaline zone in the radiated eggs than in the controls. But the difference was so slight and so difficult to measure or record that we devoted our experiments mostly to fertilized eggs.

In these we made a considerable number of measurements of the depth of the granular zone. Even if it was not seen perfectly in profile, it was possible to obtain a fair approximation by measuring from the center of the apparent ellipse which marked the surface of the granular zone. The results of these measurements showed percentages of the total diameter (inside the membrane), occupied by the granular zone, varying from 45 to 67 per cent. Twice with unradiated eggs we corroborated Heilbrunn's finding of a time of increased viscosity at 20 minutes after fertilization (1921), once on comparison with 40- and 60-minute intervals, once on comparison with 15-, 25- and 30-minute intervals. But we did not attempt to trace the natural changes of viscosity after fertilization in the strict quantitative way that Heilbrunn did. We were concerned only with guarding against confusion on our comparisons between radiated and unradiated eggs. The greatest depth of the granular zone noted, 67 per cent, was in an egg centrifuged for 4 minutes in the electric machine, 20 minutes after fertilization. The least, 45 per cent, was in an egg centrifuged electrically for 10 minutes from 23 to 33 minutes after fertilization. In a single lot of eggs the individual measurements were apt to vary over a range, for example, from 50 to 60 per cent. When it came to comparing radiated and control eggs under otherwise identical treatment, we found the average depth of the granular zone in the radiated egg in most cases slightly less than in the controls, indicating apparently a decrease in viscosity on radiation and a confirmation of Redfield's observation. But the differences were small compared with those between individual measurements, and in some instances the average measurement was greater in a group of radiated eggs than in the controls. For this reason we concluded that we could not measure the depth of the granular zone with sufficient accuracy to afford a satisfactory criterion.

The first time we mixed radiated and control eggs, and therefore saw them side by side in the same microscopic field, which was in the course of

our 9th experiment with β-radiation, we noticed a clearly visible difference between them. Whereas in the unradiated eggs the individual granules could be distinguished throughout the granular zone, in the radiated eggs there appeared at the bottom of this zone a layer of more opaque material which gave the impression that the granules there had been finely pulverized. When we examined all the eggs which lay sufficiently on their sides, this difference appeared to be universal. In the same lot of eggs the hyaline zone appeared distinctly clearer in the radiated eggs than in the controls. These eggs had been centrifuged for 15 seconds with 8985 g. 20 minutes after fertilization. In another mixed lot which was centrifuged with these, but which had been fertilized only 15 minutes previously, the same consistent difference was found; the radiated eggs showed generally the same small zone of more opaque material at the bottom. This we designated the "sediment zone." In the same experiment another pair of tubes from the same mixed lots were centrifuged with 7250 g. for 25 seconds, this time at 52 and 47 minutes after their respective times of fertilization. Both lots showed clearly the "sediment zone" in the radiated eggs, but not in the controls. In the 52-minute lot the depth of this zone was measured in some of the eggs and found to be about 20 per cent of the diameter of the egg (measured inside the membrane). This is shown distinctly in figure 2. Thereafter our efforts were chiefly centered on establishing the dependence of this "sediment zone" upon radiation, and on obtaining good microphotographs to illustrate its presence in radiated eggs and its absence in the controls.

Figure 2 shows eight radiated cells from six different experiments, all showing the sediment zone more or less distinctly. As the photographs show, it usually looks like a sediment lying in the bottom of the cell. In figure 3 are shown photographs of radiated and control eggs in pairs, each pair being taken from a single experiment in which the radiated and control eggs were mixed before being fertilized. In each case the eggs to be photographed were selected as being those in which the zones appeared most nearly in profile, and which therefore were most fairly comparable. In each case the characteristic difference appears; in the controls the granular zone seems to consist of granules of about the same size throughout, whereas at the bottom of the radiated eggs is seen the small layer of denser and apparently more finely divided material. In the third pair shown in figure 3 there is an additional difference; here the whole granular zone in the radiated egg is packed into a smaller volume in the lower half of the cell, and a perfectly clear hyaline zone is seen above it, whereas in the control the hyaline zone seems to be much smaller. On this latter point we cannot be altogether certain. In the control, granules appear to be thinly distributed as high in the cell as the bottom of the oil drops, but this may be due to a biased zone-formation: i.e., the center of the oil

drops may not be directly opposite the center of the granular zone, so that while the oil drops appear in profile, the granular zone is seen obliquely. We do not think that this can account for all of the difference; the pictures strongly suggest a closer packing of the granular zone in the radiated egg. Thus a difference in viscosity appears in this comparison, and lends support to Redfield's observation.

In figure 4 is shown a field including several radiated eggs and a few controls. The sediment zone can be seen distinctly in four out of seven radiated eggs photographed with the zones approximately in profile; in two more there is a suggestion of it; in one it does not appear. In the control eggs, of which four and a half appear in the figure, we found no suggestion of a sediment zone.

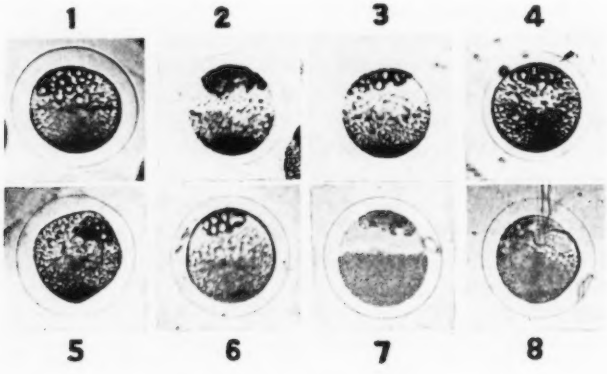

Fig. 2. Radiated eggs centrifuged after fertilization, showing sediment zone.

1, Radiated with 39 m.c. (millicurie centimeters) for 30 minutes, 4 hours and 10 minutes before fertilization. Centrifuged with 8985 g. for 24 seconds, 35 minutes after fertilization. Photographed about 1 hour after fertilization. July 21, 1919.

2, *3*, *4*, Radiated with 51 m.c. for 16 minutes, 1 hour and 53 minutes before fertilization. Centrifuged with 8985 g. for 24 seconds, 30 minutes after fertilization. Photographed 43 minutes after fertilization. July 22, 1919.

5, Radiated with 51 m.c. for 16 minutes, 2 hours and 28 minutes before fertilization. Centrifuged with 9770 g. for 23 seconds, 45 minutes after fertilization. Photographed 1 hour after fertilization. July 22, 1919.

6, Radiated with 82.6 m.c. for 10 minutes, 2 hours and 13 minutes before fertilization. Centrifuged with 11380 g. for 14 seconds, 40 minutes after fertilization. Photographed 1 hour and 7 minutes after fertilization. July 23, 1919.

7, Radiated with 82.6 m.c. for 10 minutes, 40 minutes before fertilization. Centrifuged with 8985 g. for 24 seconds, 46 minutes after fertilization. Photographed 55 minutes after fertilization. Ju'y 23, 1919.

8, Radiated with 69 m.c. for 20 minutes, 40 minutes before fertilization. Centrifuged with 7640 g. for 26 seconds, 30 minutes after fertilization. Photographed 1 hour after fertilization. July 24, 1919.

This photograph was taken 77 minutes after fertilization and 30 minutes after centrifuging. It is of interest that most of the unradiated eggs had by this time undergone cleavage (this is apparent in two or three of those in the figure), whereas none of the radiated eggs showed cleavage (cf. Packard, 1915). In three lots of eggs we found this difference; two were in one experiment in which radiation was with 39 millicurie-cm. for 30 minutes (1167 m.c.m.) the intervals between fertilization and observation

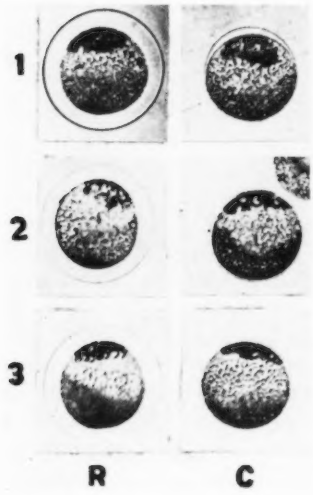

Fig. 3

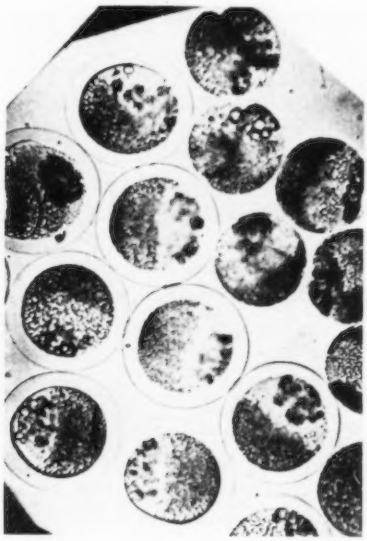

Fig. 4

Fig. 3. Radiated and control eggs in pairs for comparison, one of each from the same experiment: *R*, radiated; *C*, control.

1, Radiated *R* with 51 m.c. for 16 minutes, 2 hours and 23 minutes before fertilization. Both radiated and control eggs centrifuged with 8985 g. for 24 seconds, 25 minutes after fertilization. Photographed 35 minutes after fertilization. July 22, 1919.

2, Radiation same as in *1*. Centrifuged with 9770 g. for 23 seconds, 45 minutes after fertilization. Photographed 1 hour after fertilization. July 22, 1919.

3, Radiated *R* with 69 m.c. for 20 minutes, 30 minutes before fertilization. Centrifuged with 7640 g. for 26 seconds, 40 minutes after fertilization. Photographed 55 minutes after fertilization. July 24, 1919.

Fig. 4. Several radiated and control eggs in the same field with the sediment zone distinct in four of the radiated eggs.

Radiated with 82.6 m.c. for 10 minutes, 2 hours and 8 minutes before fertilization. Radiated and control eggs centrifuged with 9550 g. for 32 seconds, 47 minutes after fertilization. Photographed 77 minutes after fertilization. July 23, 1919.

being 87 and 82 minutes; the third lot was radiated with 82.6 millicurie-cm. for 10 minutes (826 m.c.m.) and observed 77 minutes after fertilization.

In the first few experiments after the sediment zone was first noted, it appeared to be an almost universal occurrence in the radiated eggs, and was never found in the controls. In some of the later experiments, however, it failed to appear in the radiated eggs, and in other experiments in which it did appear in them there was also a slight suggestion of it in the controls. But it never was as distinct in the controls as in the corresponding radiated eggs, and not nearly as well defined as in the eggs shown in figures 2, 3 and 4. In some experiments, although we did not find the typical sediment zone in the radiated eggs, we did notice that the entire granular zone had a more opaque and pulverized appearance than in the controls.

We have sought to find the conditions which determined whether the sediment zone appeared or not, and the conditions which in some experiments caused the appearance of the diffuse opacity of the granular zone, just mentioned. Forty-six double lots of eggs (R and C mixed) were examined, these being subdivisions of nine experiments; i.e., there were nine different radiations, and portions of the radiated eggs were mixed with controls for fertilization and centrifuging in forty-six lots. In most cases the eggs were from freshly cut worms, but in a few experiments we used eggs that had been shed. This may have introduced differences in the initial state of the protoplasm, a circumstance that would obscure the comparison of the controlled factors of radiation, centrifuging and time. Consequently our data do not suffice to determine with certainty the conditions for the appearance of the sediment zone. But examination of our data, when tabulated, brings out the following points: There were five lots in which a suggestion of a sediment zone appeared in control eggs; these were strongly centrifuged from 25 to 46 minutes after fertilization. The sediment zone became well defined only with radiation of 800 millicurie-centimeter-minutes or more. Several lots radiated with 250 m.c.m. failed to show it at all. Small sediment zones appeared in two lots radiated with only 261 m.c.m., but these were among those lots in which the controls also showed a suggestion of this zone. The result may therefore be partly ascribed to strong centrifuging at a favorable time after fertilization. Several lots radiated with slightly more than 800 m.c.m. showed well-defined sediment zones, but it was most marked when the radiation exceeded 1000. The time elapsing between fertilization and centrifuging did not seem to be a crucial condition, for among the experiments in which the effect was conspicuous this interval varied from 7 to 52 minutes. Three lots centrifuged at an interval of 15 minutes after fertilization seemed to show less distinct sediment zones than similar lots centrifuged after longer or shorter intervals. Thus there seemed to be, at 15 minutes

after fertilization, a time when the tendency to form this zone was at a minimum, but of course it would require more observations to be certain of this point. Diffuse opacity of the granular zone of radiated eggs, as compared with controls, appeared with less radiation than was required to produce a distinct sediment zone, and was found most commonly in eggs centrifuged more than 40 minutes after fertilization, but occasionally when the interval was as short as 20 minutes. The amount of centrifuging did not seem to be critical, as long as it was adequate. Centrifuging with 7000 g. or more for 10 seconds or more sufficed to produce the sediment

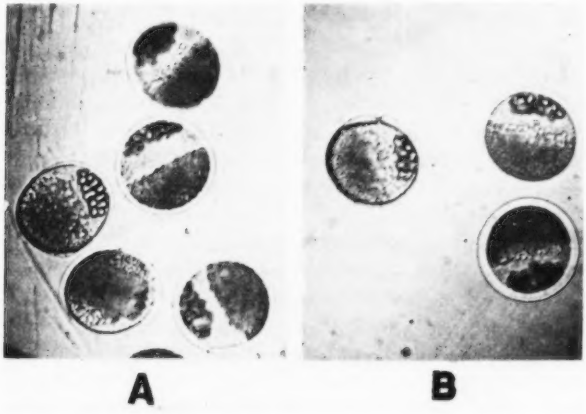

Fig. 5. Two groups of radiated and control eggs in the same field illustrating changes in granular zone after moderate radiation. July 24, 1919.

A, Radiated with 35.7 m.c. for 7 minutes, 16 minutes before fertilization. Centrifuged both radiated and controls with 10300 g. for 70 seconds, 25 minutes after fertilization. Photographed 37 minutes after fertilization.

B, Radiated with 35.7 m.c. for 7 minutes, 3 hours and 13 minutes before fertilization. Centrifuged both radiated and control eggs with 9370 g. for 22 seconds, 20 minutes after fertilization. Photographed 42 minutes after fertilization.

zone, provided other conditions were right. More than enough centrifuging seemed to make little difference, unless it caused the appearance of a small sediment zone in unradiated eggs, when done more than 25 minutes after fertilization.

Figure 5 A shows a group of eggs, including three radiated and two controls, which illustrate the denser packing of the granular zone after radiation. In two of each the zones are seen nearly enough in profile to offer a fair comparison, and although two eggs are slightly out of focus, the figure leaves little doubt that in the radiated eggs there is a sharper line of separation between the granular and hyaline zones, suggesting a closer

packing of the granules. This difference was generally found in the lot of eggs from which this picture was taken, and in most of the eight lots in this same experiment. The radiation in this experiment was only 250 m.c.m., which was less than in the experiments in which a sediment zone was produced; the relatively slight enlargement of the membrane is an indication of the small amount of radiation.

Figure 5 B, from another lot in the same experiment, shows one radiated egg and two controls. This brings out quite clearly the diffuse opacity of the radiated granular zone as compared with the controls; the granular zone appears darker and more homogeneous, individual granules being less easy to distinguish. This difference, which seemed to be as generally found in this experiment as the difference in surface packing of the granules, was also found in several other experiments with stronger radiation. Both greater opacity in the granular zone and closer packing of the granules at its surface, seem to be most easily explained, in accordance with Redfield's suggestion, as due to decreased viscosity after radiation.

It is a peculiarity of Nereis that the material is only available during the wane of the moon. All of our experiments were done in the July and August moons. The experiments with radiation were done in July. By the August moon an accident had occurred to the radium emanation pump, and we were unable to obtain any source of radiation. Fortunately we had obtained several good photographs showing the sediment zone, and our material was well employed in making a large number of control experiments to see if this sediment zone might not be found under certain conditions in unradiated eggs, and in comparing the zone-formation at different times after fertilization.

The chief result of these August experiments was to show that the well-defined sediment zone, such as we found after radiation (figs. 2 to 4), never appeared in unradiated eggs. We also failed to find such close packing of the granules as is shown in the radiated eggs in figure 5. Occasionally a single egg was found in which this packing was very marked, but one of these, at least, subsequently showed signs of cytolysis and was probably in an abnormal condition. In general, radiation seemed clearly to increase the separation of the zones, suggesting a decrease in viscosity, and to be definitely the cause of the change resulting in the sediment zone.

Discussion. We set out to search for evidence of a change in viscosity of the cytoplasm upon radiation. The denser packing of the granular zone into the bottom of the cell and the greater clearness of the hyaline zone, which usually followed radiation, seem to be most easily explained as evidence of decreased viscosity. But our most striking result was not the denser packing of the granular zone as a whole, but the appearance

of the sediment zone at the bottom of the granular zone. This and the more diffusely opaque appearance of the granular zone were more generally found than any marked difference in the space occupied by the granular zone.

The question arises as to what this sediment zone consists of, and whether it is simply another indication of decreased viscosity. It is conceivable that the finer particles which make up the sediment zone exist normally throughout the cytoplasm, and only when viscosity is enough reduced do they find their way to the bottom of the cell in sufficient quantity to appear as a definite zone. But the comparison of the radiated and control eggs suggests that perhaps some part of the granular material has been broken up, and the heavier portion enabled to settle to the bottom. For in the unradiated eggs the granular zone seems to be made up entirely of strikingly uniform granules of a relatively clear and refractile appearance. The production after radiation of more uniform-looking and opaque material suggests a chemical change in the granular portion of the cytoplasm.

At the suggestion of Dr. Margaret Lewis, we endeavored to stain the cells with neutral red to ascertain whether the mitochondria, for example, were the elements that composed the sediment zone. But lack of time and the failure of our supply of radium emanation prevented our following this part of the investigation far enough to obtain results.

Turning to the theoretical significance of the observed changes caused by radiation, let us first consider the apparent change in viscosity. We should expect β-radiation to exert some influence on the ionization of electrolytes in such a system as protoplasm. The system is so complex and so little understood that it would be futile at present to hazard a guess at the precise nature of the change, but, broadly speaking, two possibilities may be mentioned: 1, discharging of charged protein ions by a direct or indirect action of electrons; 2, a chemical change or denaturation of certain cellular components. In either way a decrease in viscosity might conceivably be explained. The change in the appearance of the granular zone, and especially the production of the sediment zone, tend to support the second possibility. Clark (1922) has shown changes in egg albumin on ultraviolet radiation, ascribing them to effects of electron emission on the amount of aggregation of colloidal particles, Redfield (1921) has shown that the action of α-, β-, γ- and ultraviolet rays, in causing jelly-retention, is essentially the same, and has given reasons for ascribing this action to the ionizing tendency of the rays (Redfield and Bright, 1924); (Redfield, Bright and Wertheimer, 1924). The changes we have found may thus bear a significant relation to those described by Clark.

SUMMARY

Nereis eggs radiated with β-rays, fertilized and then centrifuged from 7 to 65 minutes after fertilization, show slightly sharper separation of the resulting zones than is found in unradiated controls. This difference is most easily explained as due to decreased viscosity of the protoplasm in consequence of radiation.

When the radiation amounted to more than 800 millicurie-centimeter-minutes (sometimes less), there usually appeared at the bottom of the granular zone a small layer of more opaque and apparently more finely divided matter; this we have designated the "sediment zone." It was never clearly visible in unradiated eggs. Sometimes, especially with weaker radiation, no sediment zone was found, but the entire granular zone appeared more opaque and more finely divided than in the controls, suggesting a change of the granules similar to that which probably caused the formation of the sediment zone.

The change in viscosity is probably significant in connection with the ionizing tendency of the rays. The sediment zone suggests a chemical change or denaturation of some of the cellular components, but may perhaps be explained wholly as a result of decreased viscosity.

We wish to thank Dr. A. C. Redfield and Miss E. M. Bright for the opportunity to coöperate with them in the use of facilities and supplies. We are indebted to Dr. R. B. Greenough and Dr. William Duane of the Cancer Commission of Harvard University for placing a supply of radium emanation at our disposal. We wish to thank Doctor Redfield and Dr. L. V. Heilbrunn for advice and helpful criticism, and Miss A. Hopkins for assistance in preparing the paper for publication.

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ON THE PRESENCE OF INSULIN IN DESICCATED BEEF PANCREAS

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The experiments which are reported here were undertaken in order to determine the effect of desiccation on the insulin content of beef pancreas. For this purpose assays were made on fresh material and on corresponding amounts of the desiccated product. The method employed for drying the glands was one which was believed would alter the insulin content to a minimum extent.

METHODS. The glands were obtained directly from the slaughtered animal, trimmed free of fat and connective tissue, and passed twice through a meat grinder having a plate with $\frac{3}{16}$ inch holes. A representative sample (1 kgm.) was removed and the process of extraction begun. The remainder was spread on trays in layers of about $\frac{1}{4}$ inch thickness, and dried in vacuum ovens (15 mm. Hg). The maximum temperature in the air space was about 40°C. Desiccation was generally complete in four hours.

The method of Somogyi, Doisy and Shaffer (1924) was used for obtaining the insulin from both fresh and dried glands. We were not able to obtain as large a yield as these authors. After the alcohol and acid were added the entire mixture was stirred for three hours, left over night and filtered through paper, the residue being pressed and filtered. The combined filtrates were evaporated at 30°C. by means of an electric fan and filtered. The precipitate obtained with ammonium sulphate was dissolved, reprecipitated with ammonium sulphate and redissolved by adding 0.1 N NH_4OH to pH 6 to 8, the solution centrifugated and the clear portion, after diluting with water, treated with acetic acid to bring the reaction to pH 5. The precipitate was washed with water at pH 5 and dissolved in 1.5 cc. 0.1 N HCl, and made up to 15 cc. with water.

The dried material, after removal from the trays, was used either with or without preliminary grinding to 40 mesh. An amount (260 grams) equivalent to a kilogram of fresh material was taken, the evaporated water replaced and extraction begun. Defatting the desiccated pancreas with gasoline lengthened the time required for extraction very considerably. No insulin could be recovered from such samples when the period

of stirring was three hours. Replacement of the fat made it possible to extract within this time. This observation may have some relation to the fact that most workers secure better yields of insulin from fresh pancreas when using fat solvents for extraction, such as alcohol, rather than acid water although insulin in purified form is quite soluble in the latter.

The amounts of insulin extracted from fresh and dried material were compared by injecting subcutaneously into white rats. The animals were kept on a diet consisting of wheat, cracked corn, bread, milk, vegetables and occasionally some cooked meat. For the 24 hours preceding an assay they were deprived of all food except water. The symptoms evoked by the injection of insulin into the white rat have been described by Voegtlin, Dunn and Thompson (1924). They are depression, weakness, salivation and coma, with occasional convulsions. These effects have all been observed during the investigation here recorded. The increased effect of insulin on animals kept at higher temperatures, reported by Voegtlin and Dunn (1923), has also been noted. The quantities injected were graded with the object of eliciting symptoms varying from slight depression to coma or convulsions. The varying responses were marked slight depression, serious depression, slightly ill, seriously ill, and coma or convulsions. Although this method is subjective, except where coma or convulsions is produced, with practise, satisfactory comparisons can be made. In order to conserve space only a portion of these assays is recorded below.

To give the data a more quantitative aspect, the percentage mortality caused by the injection of the same volume of the two samples of insulin comprising a single experiment, was measured. The rats employed for a determination of this kind were divided into two groups of similar weights. Because we did not use an incubator to keep the temperature at which experiments on different days were performed, the same, occasionally apparently inconsistent results were obtained. However, injections of insulin from fresh and from desiccated pancreas were made simultaneously in order that the effect of climatic conditions might be the same for both groups of animals.

Results and discussion. The original 15 cc. containing all the insulin from a given batch of either fresh or desiccated pancreas was diluted before injecting. The even numbered preparations were made from fresh glands, the next higher odd numbered ones from the corresponding dried material. In all but the mortality experiments, whenever a rat developed coma or convlusions, glucose was injected to save the animal. In the mortality experiments the object was to record the percentage of rats which died, when deprived of glucose injections.

Preparations A-6 and A-7. Out of seven rats (100 to 129 grams)

injected with 0.50 cc. each of a 1 to 5 dilution of the original solution of insulin (A-6), four died. Out of an equal number of rats (95 to 140 grams) injected with the same volume of solution of insulin (A-7), three died. Slight depression was caused by 0.10 cc. of a 1 to 80 dilution by both preparations. Convulsions were produced by 0.20 cc. of a 1 to 20 dilution of A-6 and by 0.30 cc. of the same dilution of A-7.

Preparations A-8 and A-9. Out of nine rats (66 to 95 grams) which received 1.0 cc. each of a 1 to 5 dilution of the solution of A-8, two died. Out of an equal number of rats (60 to 98 grams) which received 1.0 cc. of a 1 to 5 dilution of A-9, eight died. Injections of 0.30 cc. of a 1 to 10 dilution of the A-8 and A-9 solutions made the rats slightly sick (115 to 153 grams) while 0.40 cc. of 1 to 5 dilution of either preparation produced convulsions (92 to 98 grams).

Preparations A-10 and A-11. Six out of ten rats (50 to 82 grams) which received 0.50 cc. each of a 1 to 10 dilution of the original solution of A-10 died. Seven out of ten (55 to 78 grams) died from a similar injection of A-11. Doses of 0.10 cc. of either preparation in a dilution of 1 to 20 failed to elicit symptoms in rats weighing between 115 and 134 grams. Injection of 0.30 cc. of a 1 to 10 dilution of either solution caused convulsions (114 to 116 grams).

Preparations A-14 and A-15. Of ten rats (74 to 98 grams) receiving 1.0 cc. of a 1 to 5 dilution of A-14 solution, eight died. Of an equal number (74 to 95 grams) receiving 1.0 cc. of 1 to 5 dilution of A-15 nine died. Serious depression was produced by 0.20 cc. of a 1 to 20 dilution of either preparation (109 to 119 grams). Convulsions were caused by 0.30 cc. of 1 to 5 dilution of the solution of A-14 and by 0.40 cc. of 1 to 5 dilution of the solution of A-15. All injections were made subcutaneously.

A large number of assays were done which have not been recorded. These gave results intermediate between slight depression and convulsions, such as serious depression, slightly sick and seriously sick, and gave additional evidence of the kind presented above.

The similarity in severity of symptoms produced by injecting equal volumes of the two preparations indicates that the quantities of insulin extracted are about the same and presumably that the amounts actually present are likewise about the same.

It was thought at one time that part of the effects produced by injections of the insulin extracted from the dried glands might be due to some toxic product formed during the process of evaporation. However, this is not likely to be the case because the convulsions or coma were just as quickly relieved by glucose in rats receiving the dried gland insulin as in those receiving the fresh gland preparation.

The retention of insulin in the dried glands suggests the possibility of

utilizing this material as a starting point in cases where it is desired to compare the yields obtained by different methods in different laboratories or in the same laboratory at different times.

CONCLUSION

By means of assays on fasting white rats it has been found that beef pancreas, desiccated in the described manner, contains approximately as much insulin as a corresponding amount of fresh material.

It is suggested that the material can be used as a convenient starting point for comparing yields of insulin in different laboratories or in the same laboratory at different times.

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THE REGULATION OF THE FLOW OF BILE

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The physiology of the gall bladder and the so-called resistance of the sphincter of Oddi have attracted considerable attention largely from a surgical point of view. Because of the pathology often encountered in the gall bladder and ducts, surgeons have been forced to make decisions regarding the functional significance of these structures and perhaps necessarily to draw conclusions with respect to the mechanism involved in the flow of bile into the intestine. The most recent reviews of the literature are those by Mann (1924a) and Chiray and Pavel (1925). I shall only mention here a few papers that embody the more or less common findings.

The anatomy of the biliary tract has been studied by Oddi, Hendrickson, Mann, and Auster and Crohn. Oddi (1887) found in the dog near the mouth of the duct a muscular ring which after separation from the fibers that join the intestinal musculature could be considered as an independent sphincter. Hendrickson (1898) found some muscle fibers running around the end of the duct but for the most part the fibers along the duct were continuous with the intestinal musculature. Mann (1920), who studied several species of animals, was able to make out a definite arrangement of muscle fibers which might function as a sphincter. Auster and Crohn (1922) did not see a muscular bundle formation that might be called a sphincter. Microscopic observations upon preparations made from dogs and man indicated that the muscle fibers at the mouth of the common duct were "scanty, widely separated and diffuse and at no time continuous." They gained the impression that the muscular apparatus of the papilla is made up of a fusion of the fibers of the intestinal musculature with the corresponding layer of the duct.

Oddi (1888) found this sphincter offered a resistance of 50 mm. Hg to fluids entering the lumen of the intestine. A resistance has been estimated and attributed to the sphincter by many observers since Oddi's work. Archibald (1919) reported similar findings. Mann (1919) recorded a minimal resistance in six species of animals possessing a gall bladder of 75 to 100 mm. water. Jacobsen and Gydesen (1922) found a resistance in dogs of 90 to 210 mm. water. Cole (1925) encountered 40 to 100 mm. water resistance in dogs.

These findings vary widely and would seem to attribute to the sphincter a physiological significance out of proportion to the anatomical structure described. The presence of an uncontrolled factor is indicated. An attempt has been made, therefore, to re-study this resistance under conditions where the tonus of the intestine might be observed and considered in interpreting the results.

In the dog and for that matter in most animals including man the duct passes through the intestine in a most oblique manner. The mouth of the duct in dogs lies 1 to 1½ inches below where it makes initial entrance into the wall of the intestine. As a physical arrangement this would constitute an efficient sphincter-like mechanism depending upon the tonicity of the intestine. The duct within the intestinal wall is subject to collapse and consequently would offer a resistance to the passage of fluid in proportion to the state of tonicity of the intestinal musculature.

Dogs under ether anesthesia were used for all experiments. An attempt

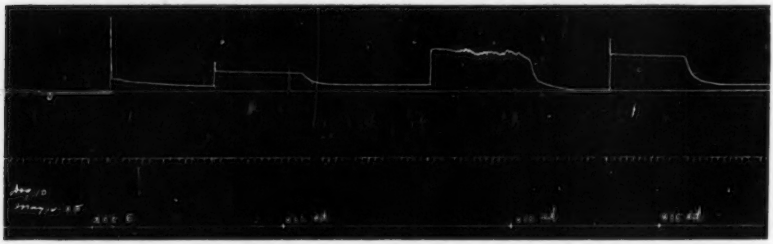

Fig. 1. Effect of adrenalin upon pressure in the common bile duct. The tonus of the intestine was increased by giving 2 cc. of 0.1 per cent eserin intravenously.

was made to insert a slender balloon through a slit in the duct into that part of the duct within the intestinal wall so that when filled with water and properly connected with a manometer the tonicity of the duct including the sphincter might be recorded. Practically, this did not work well because it was necessary to use too small a balloon. The usual technic was then utilized of placing a cannula in the duct a little distance from the intestine and pointed toward the lumen of the duct. The animal was kept under light anesthesia and the intestine well protected. The tonicity of the intestine could be observed at any time with the least amount of exposure. The cannula was connected with a manometer and pressure bottle containing water. The pressure withstood or held by the duct in a series of twenty dogs varied from 2 to 12 mm. Hg, depending upon the general condition of the animal and the degree of anesthesia.

McWhorter (1921) found that adrenalin increased the tension of the sphincter slightly. Schafer (1924) states that adrenalin causes constriction

of the bile ducts. This is in accord with Meltzer's (1917) law of contrary innervation of the gall bladder and sphincter. Figure 1 shows the typical effect of adrenalin upon duct pressure. The tonicity of the intestine has been increased by physostigmine. Adrenalin caused temporary relaxation of the intestine and the duct pressure fell to almost zero.

Atropin was used in some experiments and the atony of the intestine produced by it was effective in reducing the duct pressure as may be seen in figure 2. The recovery was less certain and consequently it did not

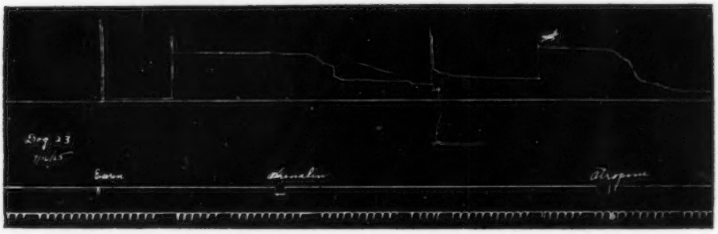

Fig. 2. Effect of atropin upon pressure in the common duct.

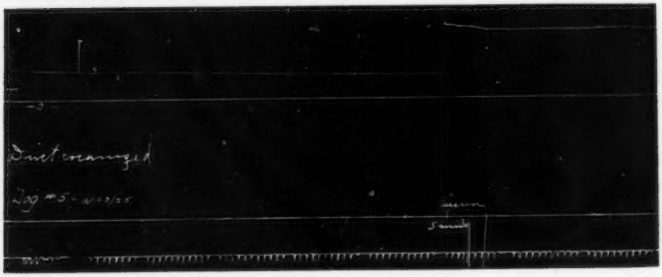

Fig. 3. After cocainizing the duct and sphincter of Oddi a resistance of 8 mm. Hg was obtained. Following the intravenous injection of eserin the resistance was greatly increased.

lend itself well when repeated trials were desired on one animal. Cole (1925) says that intravenous injections of atropin, pilocarpine and physostigmine have no effects on this resistance. His observations were made with the duodenum laid open which perhaps accounts for his results. Haberland (1924) who has recently made an extensive study of the function of the gall bladder says that atropin dilates the sphincter. However, he states that he believes that the importance of the sphincter has been over-emphasized. In the dog he thinks its action dependent upon the movements of the duodenum.

In attempting to further eliminate the possiblity of a sphincter action the duct was cocainized. Cotton was twisted about a small wire and saturated with a 4 per cent cocaine solution. This was inserted into the duct far enough that the end entered the lumen of the intestine. A second application was made and ample time allowed for the drug to become effective. The cannula was then inserted and resistance measured. Figure 3, for example, shows 8 mm. Hg pressure withstood. Following an intravenous injection of physostigmine the resistance was increased as in previous experiments where the duct was not cocainized.

Increased tonus of the duodenum brought about by mechanical irritation increases the amount of pressure that may be held by the duct. Some observers (Cole, 1925) have reported that mechanical irritation of the papilla causes a spasm visible to the naked eye. I have observed a swelling of the papilla when similarly tested but feel that it may be accounted for in part at least by contraction of the muscle fibers of the intestinal wall.

In discussing an hypothesis of the function of the gall bladder, Mann (1924b) states that the acid chyme causes a relaxation of the sphincter of Oddi and allows a flow of bile. The only observations made in this connection were upon the effect of a 0.4 per cent solution of HCl introduced into the duodenum above the entrance of the common duct. In most instances there followed a series of peristaltic waves in the duodenum. These reduced the pressure in the duct by a milking action. It seems probable that this would be an important factor in the flow of bile in digestion.

Discussion. The fact that strong inhibition brought about by adrenalin and that brought about by paralysis of the parasympathetic by atropin result equally well in lowering the resistance in the duct would indicate that if there is a reciprocal activity between the gall bladder and the sphincter such a mechanism probably does not play a major rôle in the regulation of bile flow.

All investigators agree that while $MgSO_4$ paralyzes the sphincter it also causes relaxation of the intestinal musculature.

Archibald (1919) seldom found a sphincter in dogs that was overcome by less than 600 mm. water. Thinking a tonic sphincter might cause bile to be forced back into the pancreatic duct and cause pancreatitis, he "abolished" the sphincter in dogs and found the resistance reduced to 70 mm. water or less for as long as eight weeks after the operation. McWhorter (1921) attempted to bring about the same end by dilating the sphincter. He says the sphincter was paralyzed by a quite severe dilatation (a small hemostat was used). The resistance was reduced from 180 to 200 mm. water to 25 to 50 mm. water. Of course in such experiments severe damage must have been done to the duodenum and the duct within

its walls. The extent of injury and the time element would be important factors to be considered in determining the cause of the reduced resistance.

Judd and Mann (1917), in a paper on the effect of cholecystectomy, say that following the removal of the gall bladder in the dog or cat the ducts dilated within 60 days. They cite a typical experiment (expt. 469) whereby 113 days after removal of the gall bladder in a dog the common bile duct increased in diameter from 6 mm. to 1 cm. but after entering the intestinal wall the diameter was still 6 mm. They set about to study the mechanism that produced dilatation of the ducts after cholecystectomy. The part played by the sphincter was investigated by three methods: 1, by comparing the pressure withstood by the sphincter in animals before and following the removal of the gall bladder; 2, at the time of removal of the gall bladder, by dissecting the duct free from the muscle fibers of the duodenal wall; 3, by sectioning the mucosal opening and a portion of the sphincter through a duodenal incision. Regarding the first point, they always found the residual pressure after removal of the gall bladder much lower than in normal animals. No explanation is offered. Further study may throw light upon this point. Concerning the second point they say, "In those animals in which the muscle fibers were dissected free from the intramural portion of the duct at the time the gall bladder was removed, dilatation of the duct did not occur except when there was mechanical obstruction due to adhesions." This would point strongly toward the seat of the resistance lying in that portion of the duct within the duodenal wall. Judd and Mann did not see this interpretation, as we shall see later.

"Section of the mucosal opening and a portion of the sphincter through a duodenal incision after the removal of the gall bladder has given variable results in regard to the effect on the duct and positive conclusions cannot be drawn. However, in one perfect experiment the same dilatation occurred as in animals in which the gall bladder had been removed." This would again indicate the insignificance of the sphincter. They give these findings a different interpretation. In summarizing they say in part, "After the removal of the gall bladder all the ducts outside the liver dilate. The sphincter at the entrance of the common bile duct into the duodenum is the chief factor in producing this dilatation." Certainly such a conclusion is not justifiable by the experimental data presented. For the most part the results support the theory that the seat of resistance is in that portion of the duct within the wall of the duodenum.

No great tonicity of the duodenum is necessary to close the small lumen of the duct. The normal tonus is no doubt sufficient to prevent a continuous flow when the gall bladder is present. When the pressure is high enough to overcome this resistance the bile flows into the intestine. The increased mechanical pressure upon the filled gall bladder brought about

by inspiration may easily be conceived to cause bile to spurt into the intestine irrespective of considerable tonus in the duodenum. Coupled with this is the peristalsis of the duodenum in digestion that invariably causes a flow when there is a moderate pressure in the duct. This is brought about partly by a milking action on the duct and probably partly by aspiration due to reduced pressure in the duodenum following the peristaltic wave. Rost (1923) states that in cholecystectomized dogs there is a continuous flow of bile. In observing the flow in normal dogs under ether through an opening in the duodenum he observed spurts of bile absolutely synchronous with inspiration.

In emphasizing the importance of the tonicity of the intestine and intestinal peristalsis in the regulation of bile flow it is not intended to leave the impression that the author feels that the theory is entirely proven. Further evidence is necessary. It may be that the contractions of the gall bladder and the activity of the sphincter play a part. A reciprocal physiological action between the gall bladder and the sphincter has not been satisfactorily demonstrated. This study indicates that such action may not take place and suggests that intra-abdominal pressure, respiration, and tonus and peristalsis of the duodenum are the most important factors in regulating the flow of bile.

CONCLUSIONS

1. The normal tonus exhibited by the duodenum offers resistance to pressure in the common bile duct.

2. Drugs that affect the tonus of the intestinal musculature affect this resistance. Pilocarpine and physostigmine greatly increase the amount of pressure withstood in the duct while adrenalin and atropin reduce it to a minimum.

3. Peristalsis of the duodenum is an important factor in emptying the duct.

4. The function of the sphincter of Oddi has been over-emphasized and not enough attention has been given to the tonicity of the intestine in attempting to measure the resistance of this sphincter.

5. This study favors the hypothesis that bile flow is regulated by intra-abdominal pressure and tonicity and peristalsis of the duodenum rather than being dependent upon a reciprocal activity between the gall bladder and the sphincter of Oddi.

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THE CAUSE OF DEATH FROM ANOXEMIA

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Just how lack of oxygen affects the process of life in a cell has been a matter of much speculation, but the knowledge thereon is very meager. It seems a truism to state that a cell is not dead as long as it continues to respire and that death, therefore, cannot be said to have occurred until oxidation ceases. From this point of view, we may say that death of the cells of the body is always due in last analysis to interference with oxidation, whether this is brought about by lack of oxygen, by the presence of substances which interfere with oxidation, by mechanical injury, or what not. Barcroft (1) has stated that "Anoxemia not only stops the machine but wrecks the machinery." The statement is correct but can be made more comprehensive by substituting decreased oxidation for anoxemia or oxygen want. Oxygen, like money, is of no value unless we can use it. Oxidation is the important thing for the cell and oxygen want is but one of many means of reducing oxidation. Thus, hydrocyanic acid and undoubtedly many other poisons exert their effects by interfering with the utilization of oxygen. Clear statement in this difficult field is essential to clear thinking. Oxygen is required for oxidation, oxidation is essential to the cell as the source of energy, energy is required for the internal and external work of the cell. By external work we mean the specific physiological activity of the cell, in the case of the muscle cell, contraction, in the glandular cell, secretion, in the nerve cell, the discharge of the impulse, etc. By the internal work of the cell we mean, that in total absence of external work, the living cell requires energy for the maintenance of its structure, the directional work of permeable membranes, the maintenance of potential inequalities, the prevention of free diffusion. It is certain that conditions within the cell are not uniform throughout. There must be points of great concentration of certain materials in order to account for the syntheses which occur. The temperature is probably not the same throughout and diffusion is not free, as in non-living systems, but is directional. In brief, the interior of the cell is not homogeneous during life and work must be constantly per-

formed to maintain the *status quo*. The work of remaining alive, then, is a very real thing. This fact is best illustrated by the oxygen metabolism of resting seeds as long as they remain viable (2). The only work done by the resting seed as a result of this energy exchange is the work of remaining alive. Certain of these ideas have been presented by Warburg (3). A correct understanding of the process or processes through which decreased oxidation produces its damage, may result in devising methods to combat this condition, thereby enabling us to prolong life. In certain crises, such methods might lead to complete recovery.

In previous publications, Koehler, Brunquist and Loevenhart (4) demonstrated the acidotic nature of anoxemia. The demonstration of a grade of acidosis just before death from anoxemia heretofore considered as altogether incompatible with life, suggested the possibility that the acidosis *per se* might be the cause of death. The purpose of this paper is to present experiments bearing on this aspect of the problem. We desired to investigate in greater detail than has thus far been done, the mechanism by which oxygen want causes death, and especially the part played by the initial alkalosis and the final acidosis. We have studied: 1, the degree of acidosis existing at the time of death; 2, the rate of recovery from acidosis; 3, whether there is a definite degree of acidosis producible by anoxemia, which is incompatible with recovery; 4, death by anoxemia in which acidosis was prevented either by giving alkalies or by causing such rapid asphyxial death that acidosis did not have time to develop; and 5, effect of the preliminary alkalosis on the course of events.

Experimental. Pigs were used exclusively. The methods of experimentation are identical with those discussed in previous papers (5) except that for the experiments of short duration, the pigs inspired oxygen-nitrogen mixtures from a large spirometer of the Tissot type. The spirometer was fitted with a rotary air pump so that the gases could be rapidly and thoroughly mixed. A mask fitted with inspiratory and expiratory valves was so constructed that a diaphragm made of thin sheet rubber with a perforation in the center fitted tightly over the muzzle of the animal. In all the shorter experiments, and in certain of the longer ones, the femoral canal was exposed under local anesthesia. This facilitated the obtaining of arterial blood samples and the making of intravenous injection when this was desired.

The hydrogen ion concentration was determined directly on whole blood by the electrometric method and the total carbon dioxide directly on whole blood by means of the Van Slyke constant pressure apparatus. The carbon dioxide tension was determined from the Hasselbalch formula using a pK value of 6.15. Volumes per cent of free carbon dioxide were converted to carbon dioxide tension by using the solubility coefficient of 0.0587 for whole blood at 38°C. (6).

TABLE 1

Severe anoxemia

NUMBER OF EXPERIMENT	PIG	WEIGHT	CONDITIONS	DURATION OF PERIOD	O_2 RANGE	MAXIMUM RESPIRATORY RATE OBSERVED	TIME OF BLOOD SAMPLE AFTER REMOVAL FROM CHAMBER	pH	TENSION CO_2	TOTAL CO_2	REMARKS
		kgm.		*hours*	*per cent*		*minutes*		*mm. Hg*	*vol. per cent*	
1	1	17.2	Fasting, normal O_2	8		24		7.40	44.1	49.8	
	Male		Fasting, O_2 deficit	5.5	10.5 to 6.9	88					Died
2	2	8.6	Fasting, normal O_2	24		32		7.39	51.4	55.9	
	Male		Fasting, O_2 deficit	29	10.0 to 6.5	104	15*	7.16	41.2	27.2	Recovered
3	3	7.9	Fasting, Normal O_2	24		60		7.39	47.0	51.1	
	Female		Fasting, O_2 deficit	18	9.6 to 5.2	140	10*	6.98	57.1	26.2	Died, blood obtained 2 minutes before death
4	6	10.9	Fasting, normal O_2	20		55		7.41	41.8	48.1	
	Female		Fasting, O_2 deficit	6.3	11.0 to 4.8	140	3*	6.72	16.6	4.6	Bled from heart, died from hemorrhage
5	8	9.0	Fasting, normal O_2	18		45		7.40			
	Male		Fasting, O_2 deficit	21	10.0 to 5.4	178	12†	6.80			Pig changed from chamber to spirometer containing 3.0 per cent O_2 (29 minutes)
							16	6.72			
							27	6.58			
					3.0		29	6.56			Died

6	9 Female	11.0	Fasting, normal O_2 Fasting, O_2 deficit	6 48	6.0 to 5.0 Pure H_2	46 180	13† 16 24 29 31	7.41 7.18 7.08 6.75 6.70 6.70‡	46.1 6.9 4.2 26.6 35.6 33.7	53.0 4.7 2.6 7.8 9.5 9.0	Pulse rate rose from normal of 126 to 190 and during last 30 minutes gradually dropped

* Time after removal from chamber before blood sample could be obtained. Animal breathed air during this period.

† Time after removal from chamber that blood samples were obtained. In experiments 5 and 6, animal breathed 3 per cent oxygen from the spirometer, after removal from chamber, until death occurred.

‡ Taken 2 minutes after last respiration.

Severe anoxemia. The experiments recorded in table 1 were directed toward determining how severe an acidosis could be produced by anoxemia before the intervention of death. Information was also desired as to whether a certain pH value existed beyond which recovery was impossible. Long periods of anoxemia were chosen so as to obtain greater time for the establishment of equilibrium between tissue and blood, and also for all possible physiological adjustments in the respiration, the circulation, and the tissues themselves. In order to obtain the lowest levels of blood pH before death supervenes, the animal should be kept for several hours at a moderately low oxygen level (ca 8 per cent) to permit of physiological adjustments and then subjected to very low oxygen levels (4 to 5 per cent) quickly. He must then be closely watched and removed from the chamber just before complete failure of the respiration, if resuscitation is desired. At the end of the periods of exposure to low concentrations of oxygen, difficulty was usually encountered in obtaining blood samples because of the enfeebled circulation, so that the animal breathed normal air for a sufficient period of time for some recovery to take place. To overcome this difficulty, the animals in the later experiments were rapidly removed from the low oxygen chamber at the proper moment and then made to inspire from a spirometer containing a low percentage of oxygen. In this manner the femoral artery could be exposed under procaine and repeated blood samples taken by means of a small needle, without permitting the animal to breathe normal air during the period.

The lowest pH value obtained in this series was 6.56 in experiment 5 just before respiration stopped. In experiment 6 the pH value dropped to 6.70. This latter experiment shows that the total CO_2 is lowest some time before death, for as respiration is depressed there is a slight accumulation of CO_2.

Table 2 shows the changes resulting from sudden anoxemia obtained by inspiring pure hydrogen. The lowest pH value observed before the respiration stopped was 6.81 (expt. 3). This was obtained at the end of 30 minutes and demonstrates the great rapidity of acid production. That death may occur with the arterial blood on the alkaline side of normal is shown in experiment 5. This undoubtedly was due to the marked initial alkalosis, pH 7.86 (at 10.5 minutes), obscuring the influx of acid from the tissues into the blood. That the influx of acid was extremely rapid even in this case was shown by a change of 0.32 in pH value toward the acid side in the 2.5 minutes following the previous observation (at 13 minutes) when the pH had fallen to 7.54. That this acid was non-volatile and not CO_2 was shown by the approximately constant total CO_2 values over this period. In this experiment the circulation and respiration failed in 17 minutes when the blood pH was 7.44. It is thus apparent that the respiration and circulation may fail during marked anoxemia when the

TABLE 2

Sudden anoxemia in which the pigs breathed pure hydrogen from spirometer

NUMBER OF EXPERIMENT	PIG	WEIGHT	TIME	RESPIRATORY RATE	HEART RATE	pH	TENSION CO_2	TOTAL CO_2	REMARKS
		kgm.	*minutes*				*mm. Hg*	*vol. per cent*	
1	9	13.6	0	46	120	7.42	45.8	53.8	
	Male		1			7.41	36.3	41.8	Considerable struggling
			5	44	90	7.50	17.7	24.7	Convulsive movements. Comatose
			8	10	108	7.37	10.1	10.6	
			11	5	150	7.13	16.8	10.4	Muscular twitchings
			13	0	0	7.09			Blood left ventricle
2	10	18.1	0	41	138	7.40	44.5	50.1	See figure 1
	Female		2	81	132	7.48	25.5	34.0	
			3	120	180	7.59	18.0	31.1	
			4	20	240	7.56	17.8	28.3	
			5	3	10	7.47	19.8	25.9	Muscular twitchings
			8	0	12	7.32	23.7	22.1	Few convulsive movements
			10		0	7.10	48.0	28.0	Blood left ventricle
3	11	13.0	0	40	120	7.39	46.6	50.7	(Not pure H_2, leak in inspiratory valve prolonged experiment)
	Female		7	52	180	7.67			
			9	54	120	7.65	24.6	48.2	
			12	48	105	7.52	24.7	36.3	
			13.5		150	7.41	31.4	36.1	
			18	42		7.32	34.8	32.4	Respirations very labored
			20	36	180	7.27	24.3	20.4	
			26	46	120	6.86	57.0	20.4	
			30	10	150	6.81	56.8	18.5	
			40	0	124				Heart stopped in 48 minutes
4	12	9.0	0	40	80	7.38	45.6	48.6	
	Male		6	144	162	7.66	20.4	41.2	Definite tetanic contractions
			14	135	140	7.71	13.3	30.1	of abdominal and leg muscles
			16	50	180	7.42			
			22	0	20	7.03	16.6	8.4	Convulsive twitches
			26.5	0	5				
			29	0	0	6.89	19.3	7.3	Blood from left ventricle. Heart still twitching
5	13	18.1	0	24	120	7.45	45.7	57.1	
	Male		4	58	140	7.74	12.2	29.8	
			10.5	0	80	7.86	8.2	26.8	Respiration stopped in 9 minutes
			13	0	0	7.54	16.7	25.8	
			14	0	0			24.8	
			17	0	0	7.44	20.4	24.9	Blood from left heart 4 minutes after heart stopped

pH of the blood is normal or above normal. Experiment 2, which is typical, is shown graphically in figure 1. A study of the figures for respiratory rate shows that this is not an adequate criterion of the ventilation of the lungs, the magnitude of which is suggested by the marked initial alkalosis, without a very great increase in rate. Observation of the animals showed that the increase in respiratory volume was due to the increase in amplitude as well as rate.

Recovery from anoxemia. The experiments in table 3 were directed toward obtaining information as to the respective rapidity of recovery from the changes in acid-base balance, the total CO_2, and the anoxemia prostration and distress. Many experiments with pigs had already revealed the fact that the symptomatic recovery from anoxemia is very rapid. No

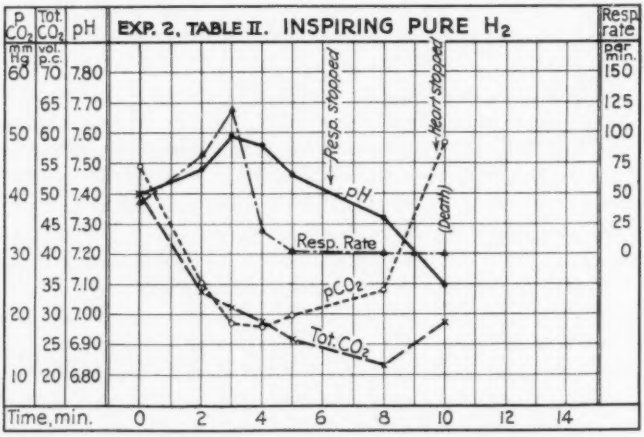

Fig. 1

matter how prolonged and severe the oxygen deficiency, if respiration could be revived at all, it was only a matter of a few minutes before the coma disappeared, and in 10 or 15 minutes the animal usually was on its feet, and in 30 minutes appeared quite normal. Experiment 3, table 3, shows a typical recovery process and is shown graphically (fig. 2). The pH of the blood recovers more rapidly than the total CO_2; in fact, a period of overcompensation results in a temporary alkalosis, usually during the second hour after the animal is placed in normal air and before the CO_2 content returns to normal. The rapid removal of the non-volatile acid from the blood increases the available alkali more rapidly than the CO_2 accumulates. The CO_2 tension, which is high in comparison with the alkali level at the beginning of recovery due to respiratory failure, gradually drops as the

available alkali for combining with it increases. It is particularly interesting to note that the total CO_2 content is slower in recovering than the blood pH.

THE PREVENTION OF ACIDOSIS DURING ANOXEMIA BY THE INTRAVENOUS INJECTION OF ALKALI. The marked acidosis encountered during severe anoxemia suggested the administration of alkali, in order to determine the part played by the acidosis in the symptomatology of oxygen want and the part it plays in the fatal outcome. In other words, we desired to observe the picture of reduced oxidation without the complicating factor of acidosis. Sodium bicarbonate was given intravenously in the form of a 3 per cent solution at a rate calculated to keep the blood pH approximately normal. The inspiration of 5 per cent oxygen from a large spirom-

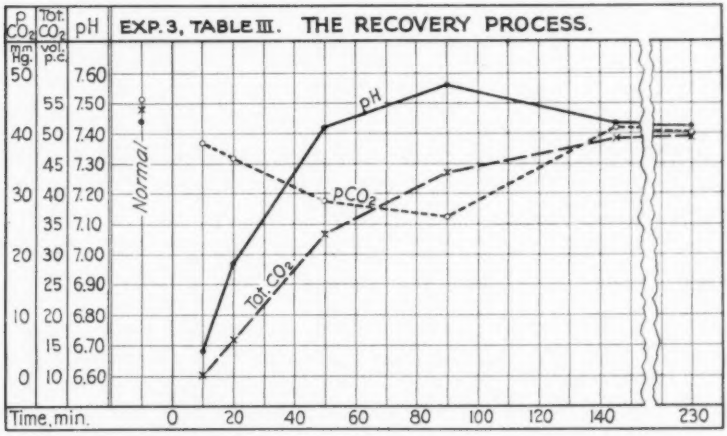

Fig. 2

eter was selected as a grade of anoxemia producing a severe acidosis within a short period of time and causing death, as denoted by cessation of respiration and circulation, in approximately 30 to 35 minutes. Previous experiments had shown that the period of alkalosis following the inspiration of 5 per cent oxygen was of a duration of about 15 minutes, so the administration of alkali was started after this period of time had elapsed and the rate of injection of the alkali was gradually increased until the end of the experiment. The administration of alkali during the period of initial alkalosis caused severe distress, marked tetany, and convulsions, with a very irregular gasping type of respiration, and was followed by complete failure of the circulation and respiration. In certain of the experiments on this subject the oxygen content of blood from the femoral artery was determined.

TABLE 3

Recovery from anoxemia

NUMBER OF EXPERIMENT	PIG	WEIGHT	CONDITIONS	DURATION OF PERIOD	O_2 RANGE	MAXIMUM RESPIRATORY RATE OBSERVED	TIME OF BLOOD SAMPLE AFTER REMOVAL FROM CHAMBER	pH	TENSION CO_2	TOTAL CO_2	REMARKS
		kgm.		*hours*	*per cent*		*minutes*		*mm. Hg*	*vol. per cent*	
1	5	10.4	Diet, normal O_2			48		7.38	45.9	49.0	
	Female		Fasting, O_2 deficit	6.8	8.6 to 5.0	154	10	7.22	51.5	38.7	
							720	7.43	42.0	50.3	
2	4	10.9	Diet, normal O_2			42		7.40	40.9	46.2	
	Female		Fasting, O_2 deficit	6.5	8.2 to 4.5	195	15	7.15	34.8	22.4	
							240	7.35	54.2	53.9	
3	5	10.9	Diet, normal O_2			60		7.42	45.5	53.8	Pig same as in experiment 1, 5 days later
	Female		Fasting, O_2 deficit	7.8	9.4 to 5.0	160	10	6.69	38.4	10.1	See figure 2
							20	6.97	36.0	16.0	
							50	7.42	28.3	33.2	
							90	7.57	26.7	43.5	
							145	7.43	41.0	49.1	
							230	7.42	40.1	49.5	
4	4	10.7	Diet, normal O_2			50		7.44	43.2	52.8	Pig same as in experiment 2, 4 days later
	Female		Fasting, O_2 deficit	6.8	9.0 to 4.4	238	16	6.94	46.5	19.5	
							75	7.36	35.5	36.6	
							204	7.54	30.0	46.2	

5	10 Female	11.0	Diet, normal O_2 Fasting, O_2 deficit	19.0	10.9 to 4.9	44 156	 12 20 60 135 190	7.41 6.61 7.21 7.42 7.49 7.41	42.1 25.2 23.2 33.2 41.8	48.5 18.5 27.3 48.1	 Bled from femoral artery exposed with aid of novocaine
6	8 Male	9.6	Fasting, normal O_2 Fasting, O_2 deficit	20.0 23.0	12.0 to 4.6	48	 7 42 135 208	7.38 6.92 7.20 7.41 7.51	45.8 42.1 22.5 25.9 28.8	48.8 16.7 16.1 29.9 41.3	

The results of the intravenous administrations of alkalies during the acidotic stage of anoxemia are given in table 5. In experiment 1, too much alkali was at first given so that an alkalosis resulted. Although life was prolonged in this experiment, as compared with controls, the marked prolongation of life was not obtained as in the later experiments (2 and especially 3 and 4) where the blood reaction was maintained at a more nearly normal level. Approximately 300 to 400 cc. of 3 per cent $NaHCO_3$ solution was found necessary to maintain the normal reaction of the blood during 80 to 90 minutes of exposure to 5 per cent oxygen. This gives an approximate idea of the amount of acid formed during this period and simulates a method of titration.

That the prolongation of life was not due to the amount of fluid injected and the copious elimination that took place through the kidneys, was shown by a duplicate experiment, no. 5, table 5, under identical conditions except that Ringer's solution was administered in similar amounts so adjusted by the addition of NaCl that the tonicity was equal to 3 per cent $NaHCO_3$ solution. In the experiments in which no fluid was given intravenously, almost complete anuria was observed; in the experiments in which the Ringer's solution fortified with sodium chloride was administered, the secretion of urine was small, while in those in which $NaHCO_3$ was given, there was a copious secretion.

The effect of alkali on the prolongation of life can best be seen in a comparison of the control experiments 6 and 7, figure 3, with the experiment shown in figure 5. It will be noted from tables 4 and 5 that the administration of alkali tended to prevent the sudden fall in the oxygen content of arterial blood. The maintenance of a normal blood pH increases the oxygenation of the blood on passing through the lungs.

Anoxemia without alkalosis. We have tried to determine whether the loss of CO_2 from the animal body during anoxemia with the resulting alkalosis is detrimental or beneficial in the life processes. *A priori*, it seemed possible that the preliminary alkalosis might be either beneficial or harmful, especially if it could be shown that acidosis played an important rôle in asphyxial death. The alkalosis might be beneficial in immediately neutralizing the fixed acids formed, or it might be detrimental by stimulating acid production in the body (7), and thus result in hastening the fatal outcome. Regardless of what the result might be, we desired to obtain the picture of the symptomatology and blood reaction to anoxemia uncomplicated by the initial and incidental alkalosis. This was done by increasing the CO_2 content of the inspired air and thereby preventing its loss during the hyperpnea. Many empirical attempts were necessary with various tensions of CO_2 in the inspired air in order to preserve an approximately normal CO_2 tension in the blood. The results of these experiments are shown in table 4 and experiment 7 is shown graphically in figure 3,

together with control experiments of the same degree of anoxemia but without CO_2. The conclusions from these experiments are discussed later.

Discussion. *The acid-base changes during anoxemia and recovery.* The changes occurring in pH, total CO_2, and CO_2 tension of the arterial blood during anoxemia and recovery are graphically displayed in the composite curves of figure 6. The diphasic nature of the pH curve both during anoxemia and the recovery process is particularly interesting. The slope of the curves during oxygen deficiency depends upon the degree of anoxemia. The idealized curves in figure 6 portray the course of events in the average experiment, in which there was anoxemia of sufficient grade and lasting a sufficient period of time. There were no experiments of this type in which the results differed in any essential regard from these curves, other than the differences in susceptibility of different individuals to oxygen want. The picture is essentially the same whether the animals are submitted suddenly to atmospheres very low in oxygen, so that the whole picture is compressed into a period of two hours, or whether the reduction of oxygen in the respired air is very gradual covering a period of two to four days.

The initial alkalosis always occurs, no matter how slight or severe the degree of anoxemia. The intensity of the alkalosis in our experiments has, in general, been directly proportional to the hyperpnea which, in turn, depends on the severity of the anoxemia. The highest pH value observed, 7.86 (table 2, expt. 5), occurred in an acute anoxemia in which pure hydrogen was breathed. On the other hand, the duration of the alkalosis is inversely proportional to the degree of anoxemia, the milder the oxygen deficiency, the more prolonged the alkalosis. In fact, our experiments suggest that an increase in the pH of the blood over normal may continue for days in very mild anoxemia. Here, apparently, the rate of production of fixed acids and the rate of their removal is such as to permit of a continuation of the alkalosis.

Blood acidosis, however, is not a constant finding in all grades of anoxemia. As mentioned above, it may not be present in mild oxygen deficiency, and again, it may not be present in very sudden complete deprivation of oxygen as obtained during the inspiration of pure hydrogen. In these cases the initial alkalosis is marked and the respiration and circulation may fail before the fixed acid produced in the tissues can compensate for the increased alkalinity of the blood due to the loss of carbon dioxide. In some of our experiments, the respiration and circulation failed under pure hydrogen while the blood pH was still above normal (table 2, expt. 5). In all our experiments excepting the few animals where death occurred too rapidly, as in complete withholding of oxygen, severe acidosis always occurred. The value of the blood pH at death from anoxemia ranged from 6.60 to 6.90 provided that the oxygen deficiency

TABLE 4

Anoxemia resulting from breathing air poor in oxygen and high in carbon dioxide

NUMBER OF EXPERIMENT	PIG	WEIGHT	DURATION OF PERIOD	INSPIRED AIR		RESPIRATORY RATE	TIME OF BLOOD SAMPLE AFTER REMOVAL FROM CHAMBER	ARTERIAL BLOOD				REMARKS
				O_2 Range	CO_2 range			pH	Tension CO_2	Total CO_2	Content O_2	
		kgm.	*hours minutes*	*per cent*	*per cent*		*minutes*		*mm. Hg*	*vol. per cent*	*vol. per cent*	
1	15 Male	11.4		20.9	0.06	42		7.40	44.3	50.0		Pig in chamber
			13 23	14.6 to 18.0	2.0 to 6.0	150	3	7.26	88.0	71.8		
2	16 Male	17.7		20.9	0.06	62		7.43	46.0	55.2		Pig in chamber
			15 05	14.0 to 19.0	1.8 to 6.2	96	4	7.10	105.5	62.5		
3	16 Male	17.2		20.9	0.06	58		7.41	43.8	50.5		Pig removed from chamber and breathing from spirometer last 5 minutes, 6 per cent O_2, 3 per cent CO_2
			8 16	10.5 to 5.8	2.2 to 4.4	120	5	7.11	63.4	37.6		
4	15 Male	11.2		20.9	0.06	48		7.39	45.9	50.0		Pig removed from chamber and breathing from spirometer last 5 minutes, 6 per cent O_2, 3 per cent CO_2
			18 35	11.1 to 4.8	2.4 to 3.9	120	5	6.93	86.8	35.9	3.9	
5	17 Male	12.4		20.9	0.06	50		7.44	43.7	53.4		Pig removed from chamber and breathing from spirometer last 5 minutes, 6 per cent O_2, 3 per cent CO_2
			10 57	12.4 to 5.4	2.1 to 4.0	108	5	6.88	71.6	26.6		

6	18 Female		16	50	20.9 11.9 to 4.6	0.06 2.2 to 3.2	40 144	5	7.39 6.97	45.5 38.0	49.4 16.9		Pig removed from chamber and breathing from spirometer last 5 minutes, 6 per cent O_2, 3 per cent CO_2
7*	16 Female	18.8		34	20.9 5.0	0.03 3.2	66 180	0 2 4 17 30 34	7.40 7.30 7.19 7.01 6.93 6.66	45.2 53.0 63.6 67.0 36.3 65.0	51.2 48.0 44.5 32.5 15.0 16.0	18.5 15.05 8.57 2.96 2.09 1.80	Death. See figure 3
8*	19 Female	7.71		36	20.9 5.0	0.03	45 120	0 7 32 36	7.39 7.58 7.06 6.89	44.2 20.2 2.8 4.0	48.6 33.8 1.5 1.5	18.20 1.68 1.29	Death

* In experiments 7 and 8, which were of short duration, the animal breathed from the spirometer, the chamber not being used. In experiment 7, the spirometer contained 5 per cent O_2, 3.2 per cent CO_2. In experiment 8, the spirometer contained 5 per cent O_2 and no CO_2.

TABLE 5

Anoxemia with intravenous administration of alkali

NUMBER OF EXPERIMENT	PIG	WEIGHT	DURATION OF PERIOD	O_2 INSPIRED AIR	TIME	RESPIRATORY RATE	HEART RATE	ARTERIAL BLOOD				TOTAL SOLUTION $NaHCO_3$, 3 PER CENT	REMARKS
								pH	Tension CO_2	Total CO_2	Content O_2		
		kgm.	*minutes*	*per cent*	*minutes*	*per minute*	*per minute*		*mm. Hg*	*vol. per cent*	*vol. per cent*	*cc.*	
1	20 Male	9.0	45	20.9	0	42	132	7.35			18.00	0	
				5.0	8	72	240	7.49			6.48	0	
					15	128	180	7.89			6.43	200	
					24	102	252	7.74			4.32	350	Voided urine alkaline
					32	40	190	7.39			1.72	500	Voided urine alkaline
					42	0	42	7.06			1.72		
					45	0	0				1.29		Death
2	22 Female	10.3	52	20.9	0	52	156	7.34	49.8	49.8	18.06	0	
				5.0	8	48	200	7.52	16.3	23.9	5.21	0	
					22	40	192	7.54	23.9	37.0	3.92	170	
					29	48	180	7.62	20.2	37.0	3.89	215	
					42	24	174	7.54	20.3	31.4		280	
					48	0	18	7.28	39.6	34.2	1.90	320	
					52	0	0	7.00	60.4	28.6	1.70		Death
3	23 Male	10.0	83	20.9	0	30	140	7.38	45.1	48.2	18.20	0	
				5.0	18	54	138	7.43	15.0	18.0	3.82	0	
					29	40	138	7.58	18.7	31.3	2.51	95	
					36	42	168	7.66	16.4	33.2	2.50	260	Voided, urine neutral
					43	54	150	7.61	18.5	33.0	2.80	300	Voided, neutral urine

					60	42	156	7.59	21.9	37.8	2.51	400	Voided, urine neutral
					70	30	96	7.44	25.6	31.3	2.04		
					76	0	96	7.31	34.1	31.3	0.60		Catheterized, urine slightly alkaline
					83	0	0						Death
4	25 Male	15.0	92	20.9	0	48	120	7.41	45.4	52.3		0	
				5.0	10	130	196	7.56	16.0	27.2		0	
					20	144	230	7.38	18.6	19.8		0	
					32	120	190	7.44	18.8	23.0		20	Catheterized specimen, urine neutral
					48	80	172	7.40	25.1	28.4		165	Catheterized specimen, urine neutral
					65	64	120	7.38	23.7	25.3		200	Catheterized specimen, urine neutral
					79	22	90	7.44	29.5	36.1		295	Catheterized, urine slightly alkaline
					85	0	72	7.32	33.3	31.0		335	
					92	0	0	7.28	34.7	30.0		380	Catheterized, urine neutral. Death. Figure 5
5	24 Male	10.0	35	20.9	0	42	150	7.42	42.6	50.0	18.61		Control experiment with modified Ringer's solution. 300 cc. given
				5.0	10	114	144	7.56	5.2	14.1	5.53		
					21	54		7.35	7.7	6.2	3.01		Catheterized, urine acid
					27		140	6.96	2.1	0.9	1.60		Catheterized, urine strongly acid
					33	36	108	6.75	2.7	0.8	1.22		
					35	0	0	6.72	2.9	0.8			Death. Figure 4
6	19 Female	7.71	36	20.9	0	45		7.39	44.2	48.6	18.20		Control experiment, no intravenous injection given.
				5.0	7	120		7.58	20.2	33.8			
					32	90		7.06	2.8	1.5	1.68		
					36	0		6.89	4.0	1.5	1.29		Death. Figure 3

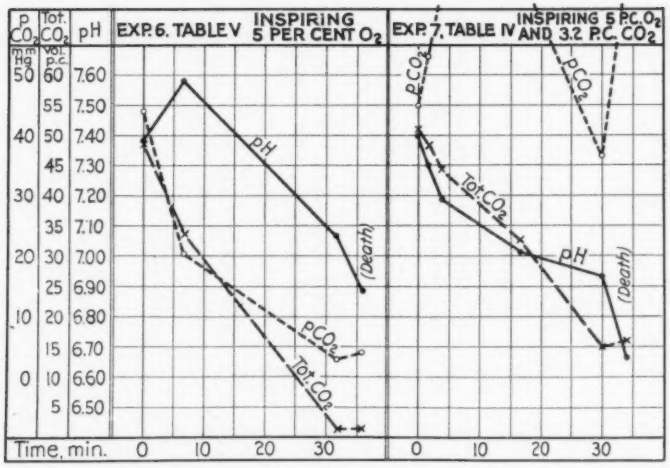
P CO2 mm Hg
Tot. CO2 vol. p.c.
pH
EXP. 6. TABLE V INSPIRING 5 PER CENT O2
EXP. 7. TABLE IV INSPIRING 5 P.C. O2 AND 3.2 P.C. CO2
pH
pCO2
Tot. CO2
(Death)
Time, min.
0
10
20
30

Fig. 3

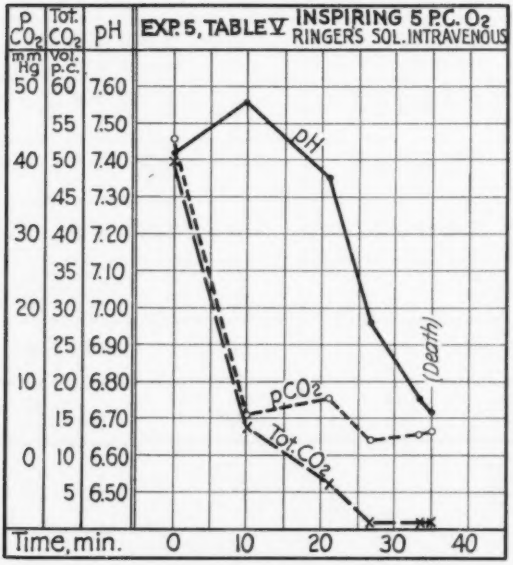
P CO2 mm Hg
Tot. CO2 vol. p.c.
pH
EXP. 5, TABLE V INSPIRING 5 P.C. O2 RINGERS SOL. INTRAVENOUS
pH
pCO2
Tot. CO2
(Death)
Time, min.
0
10
20
30
40

Fig. 4

had been of sufficient duration before death supervened. In this connection, it is interesting to note that this also was the pH of tissues immediately upon death produced by shock, as noted by Koehler (8).

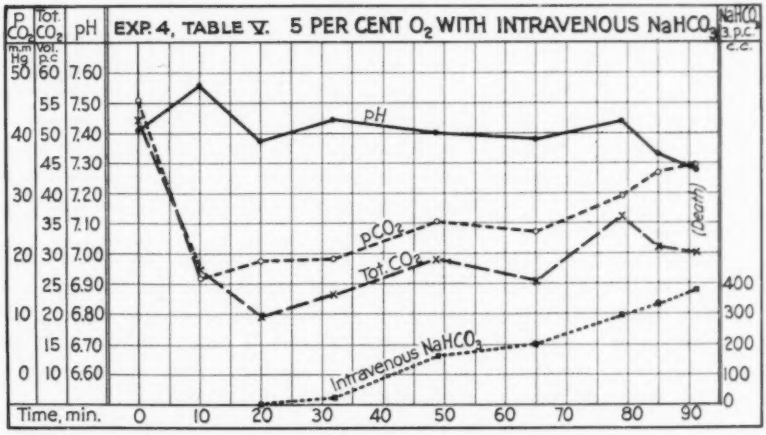

Fig. 5

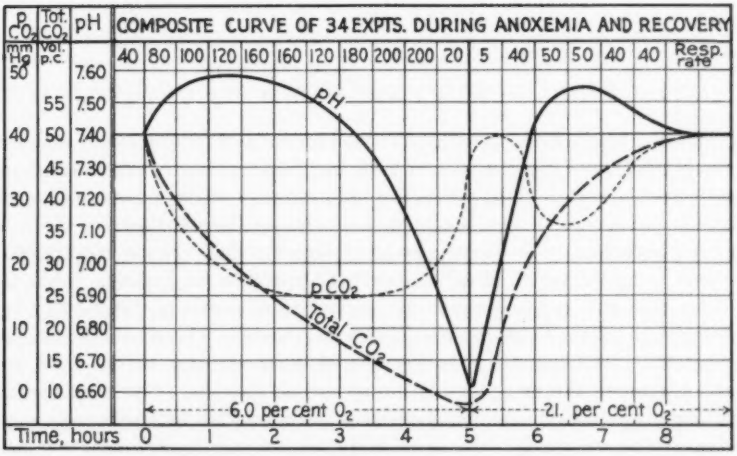

Fig. 6

The total carbon dioxide during anoxemia rapidly fell and we have observed levels as low as one volume per cent (table 5, expts. 5 and 6), this decrease having been due to loss by hyperpnea and to the neutraliza-

tion of blood alkali by non-volatile acids. As death was approached and respiration failed some minutes before the circulation, an accumulation of carbon dioxide sometimes raised the total carbon dioxide level. This increase, however, was very slight due to the very small amount of carbon dioxide produced at this stage of anoxemia.

The symptomatic recovery from the coma of anoxemia is exceedingly rapid. The animals were on their feet and behaving quite normally within thirty minutes. As shown in figures 2 and 6, the pH of the blood recovers very promptly when the animal respires normal air while the recovery of the total carbon dioxide is much slower. The blood pH usually reaches the normal within one hour and then occurs the recovery alkalosis lasting one to two hours. The alkalosis disappears only when the total carbon dioxide has returned to the normal level.

We do not know the nature of the acids occurring in the blood under anoxemia, but we do know that lactic acid occurs and that the acids disappear with remarkable rapidity on relieving the anoxemia without the production of a correspondingly large amount of carbon dioxide. If the anaerobic acid production and recovery is similar to that which occurs in muscle, we would have an explanation for the observed facts.

Meyerhof (9) idealizes the anaerobic decomposition of the hexose phosphate in muscle as follows:

$$4\ C_6H_{10}O_4\ (H_2PO_4)_2 + 8\ H_2O \rightarrow 8\ C_3H_6O_3 + 8\ H_3PO_4$$

and the oxidative recovery:

$$8\ C_3H_6O_3 + 8\ H_3PO_4 + C_6H_{12}O_6 + 6\ O_2 \rightarrow 4\ C_6H_{10}O_4\ (H_2PO_4)_2 + 6\ CO_2 + 14\ H_2O$$

According to the latter reaction, a small amount of oxygen could cause the disappearance of much acid and with the production of a minimal amount of carbon dioxide. This meets the requirements of the recovery from the acidosis of severe anoxemia and we believe that the reaction, by which the acid destruction occurs in the body of higher animals, as a whole, will be found to approximate closely Meyerhof's reaction for muscle. This may mean that the type of anaerobic acidosis and recovery, which has been found for muscle, holds for the tissues in general. The recovery alkalosis, therefore, is explained by the very rapid disappearance of the fixed acids and the much slower accumulation of carbon dioxide. The slow accumulation of carbon dioxide may be partly accounted for by hyperpnea occurring during recovery. The irritability of the respiratory center may be increased as a result of the low carbon dioxide and low oxygen pressures to which it had been exposed.

The changes of the carbon dioxide tension during oxygen want and recovery are exceedingly interesting. The initial marked drop of carbon dioxide tension and its maintenance at a low level during the greater part

of anoxemia is due to the carbon dioxide deficit caused by the hyperpnea of oxygen deficiency. Not only is it due to the direct deficit of carbon dioxide, but also to the increase in pH, a direct result of the loss of carbon dioxide, to be sure, but it had the effect of farther lowering the carbon dioxide tension because a larger percentage of what remained was fixed with alkali. During the last stages of severe oxygen deficiency, the carbon dioxide tension rose markedly in some cases. This was due to the inability of the blood to fix the carbon dioxide produced by the tissues as a result of the marked acidosis. Of the total carbon dioxide, 26.2 per cent exists as free carbon dioxide at a pH of 6.6, while only 5 per cent is free at 7.42, and 3 per cent at 7.65. During the final stage of asphyxia when respiration fails, there is an increase in carbon dioxide tension due to its retention and the absence of alkali to fix it.

The elevation of the carbon dioxide tension is maintained in the event of recovery until the respiratory process recovers. Then, a second period of low carbon dioxide tension occurs during the period of alkalosis which is noted during recovery. The secondary fall in carbon dioxide tension indicates that the blood recovers its alkalinity at a faster rate than the carbon dioxide accumulates. In our preliminary communication (10) we suggested that this period of alkalosis during recovery is due to the rapid removal of the acid products with a consequent sudden relative excess of alkali.

The prevention of carbon dioxide deficit and the resulting alkalosis during anoxemia. By increasing the carbon dioxide content of the inspired air, it is possible to avoid the initial alkalosis during the hyperpnea of oxygen deficiency. This phase of the work is of interest not only in obtaining information as to the beneficial or detrimental effect of the initial alkalosis, but also as to the suggestions throughout the writings of Yandell Henderson, that the acapnia or loss of carbon dioxide was the detrimental factor in these conditions in which such a loss occurs.

That the initial alkalosis might be detrimental is suggested by the following points:

1. The kidneys automatically tending to maintain neutrality of the blood would excrete a part of the excess of alkali (11) which would deprive the body of its use to neutralize acids during the succeeding period of acidosis.

2. The alkalosis may affect the dissociation of oxyhemoglobin sufficiently to hinder the transportation of oxygen to the tissues. Normally, this probably would not be a very important factor, but during marked anoxemia the effect of the increased pH on the dissociation is considerable.

3. Alkalosis stimulates tissues to produce lactic acid and acetone bodies (7). In thus initiating the acidotic type of metabolism, which is later a serious menace to life, the alkalosis may be detrimental.

On the other hand, it might be possible that the alkalosis during the early part of anoxemia might be beneficial because: *1*, the fixed acids undoubtedly would be more quickly removed from the tissues when the alkalinity of the blood is increased; *2*, it is probable that an increase in the pH up to a certain point is favorable to biological oxidation, whereas a decrease in the pH is detrimental (12).

Our experiments, in which carbon dioxide was added to the air breathed, although not very conclusive because of the difficulty of obtaining the proper carbon dioxide tensions, did not show that any improvement or definite change was obtained, either in the general condition of the animal or in the prolongation of its life. A typical experiment with a control experiment is charted in figure 3. It may be that in mild anoxemia, the alkalosis is favorable to the organism by facilitating oxidation, but in severe anoxemia it is entirely without effect.

The effect of preventing the acidosis of anoxemia by the intravenous injection of alkali. That the acidosis occurring during anoxemia might directly in itself be very detrimental is, of course, an obvious suggestion. There is hardly a chemical process involved in the life of the cell, surface tension, hydration, solubility, permeability, reaction rate, and so on, that is not affected by a change in reaction. It seems to be definitely established that acidosis or a decrease in the pH is unfavorable to biological oxidation. Loevenhart (13) suggested that stimulation of the medullary centers by a decrease in the pH may be readily explained on the basis that this decreases oxidation in the centers, since it is proved that decreased oxidation under proper conditions stimulates functional activity (14). It would seem, then, that we are dealing here with a vicious circle, i.e., decreased oxidation causes acidosis and acidosis further decreases oxidation, thereby making it more difficult for the tissues to utilize even the reduced amount of oxygen which is available.

The administration of alkali in our experiments was an attempt to evaluate the detrimental effect of the concomitant acidosis during severe anoxemia. Intravenous administration of sodium bicarbonate started at the beginning of the acidotic phase of anoxemia greatly prolonged the life of the animal. In the best experiments, in which the alkali was given at such a rate as to maintain an approximately normal blood pH, the animals lived 2.5 times as long as controls subjected to the same degree of anoxemia but receiving no alkali therapy. However, if the blood were made more alkaline than normal, the duration of life was again rapidly diminished.

These facts, together with the proposition that recovery from even extreme anoxemic distress with marked acidosis is exceedingly rapid as soon as oxygen is again supplied to the tissues, have led us to believe that the acidosis of anoxemia manifests its main detrimental action through a further depression of oxidative processes.

The effect of maintaining an approximately normal pH in supporting renal function under conditions of severe anoxemia is very interesting and significant, especially in the light of the work of MacNider on the value of alkali in protecting the kidney against damage from the salts of the heavy metals (15) and also from chloroform (16). It suggests a close analogy between the effect of these substances and the acidosis of anoxemia so far as the renal injury is concerned. The effect of these toxic substances may be due in large part to their power to interfere in some manner with oxidative processes in the kidney. It offers corroborative evidence of the therapeutic value in nephritis of giving alkali up to the point of neutralizing the urine. The protective value of alkali in acidotic conditions referable to decreased oxidation, as shown by the kidney, also applies to other vital functions, as shown by the increased duration of life under anoxemia when acidosis is prevented. It will surely be found that sodium bicarbonate will antagonize the toxic action of a great variety of substances,—certainly those in whose toxic action there is an element of reduced oxidation and acidosis. Hale (17) has shown that sodium bicarbonate lessens the toxicity of acetanilid. There must be many such cases.

The reactions of the organism to reduced oxidation from the viewpoint of the energy requirements of living matter. Previous publications from this laboratory (13), (14) have shown that reduced oxidation first stimulates functional activity and then, if of sufficient grade, depresses it. It has also been demonstrated that increased oxidation up to a certain point depresses functional activity (18). The reactions of the tissues of the body to reduced oxidation may be clarified by considering them from the standpoint of energy. The literature is full of loose statements due to neglect of this consideration. Various authors have stated that this or that function is affected by the oxygen percentage in the blood or the oxygen pressure in the blood. The only way a change in the oxygen percentage or oxygen pressure can affect the cells is by modifying their rate of oxidative change, that is, their rate of energy transformation. Thus, in hydrocyanic acid poisoning, regardless of the oxygen percentage or pressure, oxidation is reduced below the point compatible with life due to the fact that the cells cannot utilize the oxygen available to them. Oxygen itself is of no importance to the cells; the important thing is oxidation, and oxygen becomes important only when it effects oxidation. It is oxidation, the transformation of energy—that counts. For the transformation of energy in living matter, oxidizable material and oxygen are required. On account of extensive storage, it is difficult to affect oxidation by means of reducing the fuel supply. However, Paul Bert (19) probably succeeded in doing this by submitting animals to a pressure of ten atmospheres of 65 per cent oxygen, under which the dog had convulsions, together with a marked fall of body temperature, both of which indicated that the immediately

available combustible material had been oxidized and the animal was then suffering from reduced oxidation brought about by previous excessive oxidation. The high toxicity of powerful oxidizing agents bears testimony to the susceptibility of living tissue to excessive oxidation.

That increased oxidation of lesser grade causes depression of the respiration has been shown by Hough (20) who found that the inhalation of pure oxygen decreases the respiratory rate and the pulmonary ventilation. On the other hand, it has been shown that a decrease in the oxygen of the respired air causes an increase in the volume of air breathed. Thus Ellis (21), in a large number of observations on man, showed that there is an increase in the volume of air breathed at an average of 18.1 per cent oxygen. An analysis of the data presented by Schneider and Truesdell (22) shows that there is a definite increase in lung ventilation at 19.5 per cent. Our experience has been that an increase in volume of respiration is obtained if the oxygen percentage is reduced only a few tenths of one per cent providing the method of recording the respiration is sufficiently accurate. It is obvious, therefore, that under normal conditions, the oxygen tension of the respired air is a factor in regulating the respiration and this can only be explained by the alteration in the rate of oxygen fixation by the cell. These independent observations further support the view expressed by Bethe (23) and by Loevenhart (13) that increased oxidation of proper grade depresses, and decreased oxidation of proper grade stimulates functional activity. The mechanism of stimulation of the respiration by oxygen want has been the subject of much controversy ever since the day of Rosenthal and Pflüger. Rosenthal believed that oxygen want is the real stimulus (24). It was difficult, however, to understand how the absence of a substance could stimulate, and in order to meet this difficulty Rosenthal conceived that reduced hemoglobin must be the direct stimulus, whereas oxyhemoglobin is without such action. Pflüger (25) pointed out that reduced hemoglobin never leaves the blood stream and, therefore, could not act at a distance on the cells of the respiratory center. He maintained that respiratory stimulation by oxygen want was due to the stimulating effect of incomplete products of oxidation. This view has been widely accepted.

Haggard and Henderson (26) showed that oxygen want results in hyperpnea and an alkalosis, and hence the stimulation could not be explained on the basis of acid products. These investigators could find no satisfaction in Loevenhart's view that decreased oxidation *per se* is a sufficient explanation of the stimulation. They therefore postulate a respiratory X, an unknown substance or mechanism, as being responsible for stimulation. Yet, they have no idea of how respiratory X or any other substance results in stimulation. If their respiratory X were discovered, we would still have to attempt an explanation of its *modus operandi*.

Let us examine the explanation that oxygen want is the direct stimulus. Pflüger's criticism is invalid because the cell has previously had an ample supply of oxygen. The withdrawal of any substance necessary to cell life or modifying function is always followed by changes in function. Witness the withdrawal of morphine or any habit-forming drug from an habitué. We are habituated to oxygen,—hence, the withdrawal symptoms. Any change in the essential physical or chemical environment of the cell results in altered function, and is not withdrawal of oxygen as fundamental a change in the chemical environment as the presence of the unknown respiratory X? Could a more profound change in the chemical environment of the cell be imagined than the withdrawal of oxygen? The question naturally presents itself as to how oxygen want may stimulate. Certainly it can only do so by lessening oxygen fixation by the cell, which will reduce the energy available from this source, and the inertia of the cell in resisting this change in the rate of energy transformation may induce other chemical reactions to proceed at an increased speed. An attempt to present a conceivable explanation has been made (14), (27). The view at least has the advantage of attempting an explanation in terms of the most fundamental characteristic of living matter, namely, its power to transform energy and the inertia or resistance to change which is certainly a fundamental physical concept. According to our views, reduced oxidation directly stimulates and also induces an acidotic type of metabolism. The acidotic type of metabolism induced, interferes with oxygen fixation by the cell and thus further stimulates. This vicious circle of oxygen want and acidosis continues until oxidation is reduced to the point which can support no functional activity. Then, we have depression or paralysis. Finally, the energy liberation is reduced to a point which does not even meet the small requirement of the internal needs of the cell, referred to in our introduction. The cell is unable to maintain the *status quo* and the irreversible change, which we call death, supervenes. Acidosis and the resulting autolysis usually play a part in the disintegration of cell structure, but if oxygen is withdrawn with sufficient rapidity, death may result without acidosis. From our experiments, it appears that the duration of anoxemia or of acidosis is an extremely important factor in the production of the irreversible change.

CONCLUSIONS

The blood reaction during sufficiently severe anoxemia and during the recovery process is diphasic. In response to anoxemia, the blood shows in the first place a phase of alkalosis, due to the excessive loss of carbon dioxide during the hyperpnea. This hyperpnea is, in turn, due to reduced oxidation in the cells of respiratory center. After a certain period of time, the fixed acids produced as a result of decreased oxidation in the tissues of

the body compensate for the loss of carbon dioxide and the blood pH becomes normal. Following this, there develops a most extreme grade of acidosis, depending on the degree and duration of reduced oxidation. On restoring the animal to normal atmosphere, the coma and distress of reduced oxidation rapidly disappear. There follows a marked increase in the pH of the blood which becomes definitely alkaline, after which it gradually returns to normal.

The total carbon dioxide during the anoxemic phase is reduced to very low limits. During recovery, the accumulation of carbon dioxide is much slower than the rate of removal of the fixed acids, which accounts for the recovery alkalosis. The total carbon dioxide returns to normal much more slowly than the blood pH. The recovery alkalosis ceases with the return of the total carbon dioxide to normal. The loss of carbon dioxide (acapnia) is not of great importance in the symptoms nor the blood changes produced by anoxemia, except that it accounts for the initial alkalosis. Death from oxygen want is believed to be due to failure of the supply of energy required to maintain the physico-chemical relationships and the structure of the cell.

Death from very acute anoxemia may result while the pH of the blood is on the alkaline side of normal. In more gradual deprivation of oxygen, there develops an extreme degree of acidosis from which the animal may be resuscitated, provided measures are adopted immediately. That acidosis plays a rôle in death from anoxemia is shown by the fact that the intravenous administration of alkali during the stage of acidosis markedly prolongs life by restoring an approximately normal blood pH.

We conclude that decreased oxidation induces a vicious circle in that it causes acidosis and the acidosis further interferes with the power of the tissues to use oxygen.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

III. An Attempt to Prove that a Humoral Mechanism is Concerned in Gastric Secretion by Blood Transfusion and Cross-Circulation

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As the literature dealing with the gastric hormone question has already been discussed in our previous papers (Ivy and Whitlow, 1922), (Lim, 1922) only a brief review will be made at this time.

Edkins (1906) proposed the gastric hormone hypothesis on the basis of finding a gastric secretin (gastrin) in the pyloric mucosa. Later, with Tweedy (1909), he applied substances to the isolated, but not denervated, pylorus and obtained what was thought to be a true chemical stimulation of gastric secretion.

Popielski (1909 to 1920) and Keeton, Koch and Luckhardt (1920), however, have proved that a gastric secretin may be extracted by appropriate methods from the entire digestive tract and from other organs. A gastric secretin has even been found in plant tissues (Eisenhardt, 1910, 1911), (Kisscleff, 1912), (Miyodera, 1922), (Bickel, 1917), showing that the stimulating substance is non-specific, both as regards origin and quite probably kind (Ivy and Javois, 1925).

A number of observers (Gross, 1906), (Krzyszkowski, 1906), (Zeliony, 1913) have apparently confirmed the observations of Edkins and Tweedy (1909) on the application of "secretagogues" to the pylorus, but in none of the experiments, except those of Zeliony (1913) and Savitsch and Zeliony (1913), was intestinal stimulation unquestionably avoided. These latter investigators, using dogs with an intact pyloric pouch and a gastric fistula, observed that the glands of the fundus were stimulated on application of certain substances to the pyloric mucosa, but believed that the mechanism concerned was an enteric reflex, because stimulation from the pyloric pouch was inhibited by application of cocaine to the pyloric mucosa prior to the application of the secretagogue. Ivy and Whitlow (1922), employing dogs with a partially denervated pyloric pouch (vagi and

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enteric nerve connections with fundus being severed) and a Pavlov fundus pouch, failed to observe stimulation of the glands of the fundic pouch when "secretagogues" were applied to the pyloric pouch, probably because the pouch only was observed, whereas Savitch and Zeliony (1913) used the entire fundus. We (Lim, Ivy and McCarthy, 1925) have observed in a dog with an isolated partially denervated pyloric pouch and a gastric fistula that application of "secretagogues" to and distention of the pyloric pouch were effective stimuli, in spite of the fact that no vagal or enteric connections existed between the pyloric pouch and the fundic portion of the stomach. We proposed as a working hypothesis, a vascular mechanism of secretion involving an increased blood flow through the gastric capillary bed.

The problem has been attacked directly by Lim (1922) who transfused and cross-circulated unfed with fed animals (cats). No gastric secretion was observed except in two doubtful cases, although the intravenous injection of active gastrin, the supposed hormone, into the same animals provoked a flow of gastric juice. These experiments, however, may be criticised on two grounds, first, that the cross-circulation time was of too short a duration and the transfused quantities of blood too small, although the whole available blood of the donor was frequently transfused, and secondly, that the stimulation by the gastric hormone in the blood might normally be only minimal or subminimal, which criticisms coupled with the effect of anesthesia might mask or prevent any possible positive result. In support of this latter criticism it should be pointed out that Lim and Ammon (1923) have shown that repeated injections of subminimal doses of gastrin will eventually provoke gastric secretion. On the hypothesis that either the alleged gastric hormone is, or gastric secretagogues absorbed by the intestines, or possibly both of these agents are present in the blood in subminimal quantities and act by having an accumulative effect on the gastric glands, we have performed further cross-circulations and transfusions in unanesthetized dogs provided with gastric pouches and have extended the period of observation in the cross-circulation experiments over several days.

A. Blood transfusions. *Methods:* Gastric pouch dogs were used entirely in this work, because it was obviously important to know the state of gastric secretion in the donor as well as in the recipient.

As it is known that the blood of dogs contains iso-agglutinins (McEnery, Ivy and Pechous, 1924), it was necessary to test the dogs for compatibility. Cross agglutination tests were performed and only completely compatible dogs were used. The body temperature, heart beat, respiration and general condition of the recipient was observed at intervals before and after the transfusion to make certain that no reaction occurred. A reaction did not occur in any of our nine transfusions.

The blood was transfused by the Percy-Brown-Kimpton tube method with which method one of us (I) has had considerable experience. The transfusion was always completed in ten minutes, the animals being off the laboratory tables in fifteen minutes. From 150 to 200 cc. of blood was the amount transfused, from eight to twelve kilo dogs being used. Local procain (0.5 per cent) anesthesia was used, never more than 5 cc. being used in each dog. The blood was taken in some instances from the external jugular and in others from the carotid artery and was introduced into the recipient via the external saphenous vein.

The gastric acidity is expressed in per cent HCl in the tables.

Results: The transfusions performed can be divided in four groups: 1. A fed donor with the gastric glands of the recipient being in tone and not in tone (by tone we mean secreting free acid). *2.* An unfed donor with gastric glands of the recipient not in tone. 3. A donor injected with gastrin, or gastrin plus histamine, the gastric glands of the recipient being in tone. 4. A fed donor with the recipient being an animal with a pouch of the entire stomach and therefore a more delicate test object than a small gastric pouch.

GROUP I. *Transfusion 1.* Two and one-quarter hours after feeding the donor, 150 cc. of blood were transfused into a recipient that was secreting free acid at the time of receiving the blood. Following the transfusion there was a definite increase in the quantity of the secretion without an increase in acidity (table 1). The recipient in this case had a very large pouch, sometimes secreting 46 cc. of juice per hour after a meal.

Transfusion 3. Three and one-half hours after feeding the donor, 160 cc. of blood were transfused into a recipient that was not secreting free acid, with definitely negative results, although this animal responded excellently to a meal (table 1). This animal seldom showed a response the first hour after a meal.

Transfusion 4. Two hours after feeding the donor, 160 cc. of blood were transfused into a recipient that had been given 250 cc. of milk four hours prior to the transfusion in order to be certain that a humoral stimulus, if such exists, was exciting the gastric glands at the time of the transfusion of "fed" blood. The result was definitely negative (table 1). The recipient responded to a meal.

Transfusion 6. Three and one-quarter hours after a meal, 175 cc. of blood were transfused into a coeliacectomized recipient that was not secreting free acid at the time of the transfusion with negative results, although the animal secreted well to a meal (table 2).

GROUP II. *Transfusion 2.* This transfusion was done as an "unfed" control experiment and to ascertain if an immediate increase in the total blood volume would intensify the response to a meal. One hundred and fifty cubic centimeters of blood from an animal not secreting free acid

TABLE 1

Pavlov pouch dogs

DONOR					RECIPIENT				
Procedure	Time	Gastric secretion			Procedure	Time	Gastric secretion		
		Amount	Free acid	Total acid			Amount	Free acid	Total acid
				Transfusion 1					
	o'clock	*cc.*				*o'clock*	*cc.*		
Meal at 2:00	2–3	4.1	0.045	0.073	Continuous	2–3	3.1	0.182	0.264
	3–4	5.2	0.173	0.209		3–4	2.0	0.173	0.246
150 cc. of blood transfused to recipient at 4:15 o'clock									
	4–5	3.6	0.155	0.209		4–5	5.2	0.182	0.273
	5–6	3.1	0.091	0.136		5–6	4.9	0.173	0.246
	6–7	3.0	0.155	0.218		6–7	6.5	0.182	0.264
				Transfusion 3					
Meal	2:30–3:30	1.0	0	0.018	Continuous	1:30–2:30	1.5	0	0.018
	3:30–4:30	5.3	0.155	0.282		2:30–4:30	5.6	0	0.027
	4:30–5:30	8.0	0.419	0.455		4:30–5:30	1.0	0	0.027
160 cc. of blood transfused at 6:00 o'clock									
	6–7	9.0	0.337	0.401		6–7	3.5	0	0.018
	7–8	6.1	0.328	0.391	Meal at 8:00	7–8	3.5	0	0.027
	8–9	4.5	0.300	0.373		8–9	4.0	0	0.027
	9–10	4.0	0.282	0.346		9–10	10.1	0.145	0.228
				Transfusion 4					
Continuous	9–10	0.8	0	0.018	250 cc. milk at	9–10	1.0	0	0.054
Meal at 11:00	10–11	1.0	0	0.018	9:00 to ex-	10–11	3.7	0.191	0.328
	11–12	1.5	0	0.018	cite secre-	11–12	5.4	0.264	0.364
	12–1	8.0	0.164	0.246	tion	12–1	3.5	0.273	0.373
Transfused 160 cc. of blood at 1:15 o'clock									
	1–2	10.5	0.273	0.328		1–2	3.7	0.182	0.273
	2–3	10.2	0.319	0.410		2–3	2.0	0.173	0.273
Lapped water	3–4	13.5	0.373	0.446		3–4	1.0	0.127	0.246
at 4:00	4–5	19.5	0.401	0.492	Meal at 4:00	4–5	1.0	0.091	0.182
	5–6	13.5	0.382	0.464		5–6	7.0	0.309	0.391

TABLE 2

Pavlov pouch dogs

DONOR					RECIPIENT				
Procedure	Time	Gastric secretion			Procedure	Time	Gastric secretion		
		Amount	Free acid	Total acid			Amount	Free acid	Total acid
				Transfusion 6					
	o'clock	*cc.*				*o'clock*	*cc.*		
Meal at 1:00	1–2				Continuous	1–2	1.0	0	0.045
	2–3	15.0	0.391	0.428		2–3	2.5	0	0.045
	3–4	27.5	0.455	0.483		3–4	1.0	0	0.036
				175 cc. of blood transfused at 4:15					
	4–5	7.6	0.455	0.483		4–5	1.0	0	0.018
	5–6	3.8	0.328	0.373		5–6	1.0	0	0.018
	6–7	8.0	0.337	0.391		6–7	1.0	0	0.018
	7–8	2.8	0.209	0.246	Meat at 7:00	7–8	2.5	0.082	0.145
	8–9	4.1	0.209	0.255		8–9	8.9	0.346	0.391
						9–10	11.5	0.428	0.455
				Transfusion 2					
Continuous	1–2	2.1	0	0.027	Continuous	1–2	2.5	0	0.018
	2–3	2.5	0	0.036		2–3	1.8	0	0.018
Continuous	3–4	2.0	0	0.027	Continuous	3–4	2.0	0	0.018
				150 cc. of blood transfused at 3:40					
Continuous	4–5	2.4	0	0.027	Continuous	4–5	2.0	0	0.018
Meal at 5:00	5–6	2.5	0	0.046	Meal at 5:00	5–6	12.0	0.218	0.273
	6–7	2.0	0	0.046		6–7	14.0	0.300	0.346
	7–8	1.5	0	0.036		7–8	21.3	0.346	0.419
				Transfusion 5					
Meal at 5:10	9:30–10	4.4	0.200	0.309	Continuous	9:30–10	3.8	0.273	0.373
Meal at 5:10	10:10:30	4.0	0.273	0.364		10–10:30	5.8	0.346	0.437
Meal at 5:10	10:30–11	4.3	0.273	0.364	Continuous	10:30–11	5.7	0.300	0.428
4 cc. gastrin	11–11:30	3.8	0.300	0.373	Continuous	11–11:30	5.8	0.300	0.401
subcutaneously at 11:30	11:30–12	5.5	0.319	0.391	Continuous	11:30–12	2.3	0.237	0.364
				150 cc. blood transfused at 12:15. Took blood at 12:10					
	12–12:30	8.8	0.355	0.446		12–12:30	2.0	0.073	0.218
	12:30–1	5.7	0.355	0.446		12:30–1	3.2	0.136	0.300
	1–1:30	4.3	0.355	0.437		1–1:30	2.0	0.164	0.309
	1:30–2	3.1	0.282	0.391		1:30–2	1.5	0.110	0.246
	2–2:30	2.4	0.291	0.364		2–2:30	2.0	0.073	0.200
	2:30–3	2.0	0.200	0.273	Meal at 3:00	2:30–3	2.0	0.045	0.164
Meal at 3:00	3–4	5.0	0.155	0.209		3–4	2.7	0.045	0.145
	4–5	10.0	0.228	0.273		4–5	3.0	0.155	0.264

did not increase the secretion of the recipient, whose glands were not secreting free acid, but did intensify the response of this animal to a meal as this animal only seldom responded to a meal the first hour and never before or after, secreted 47 cc. of gastric juice in three hours. This did not occur following the other transfusions probably because a longer period than an hour and a half was allowed to lapse before the meal was given (table 2). No other control experiments were deemed necessary because of the negative nature of this and the other experiments.

GROUP III. The transfusions in this group were done in order to test or prove whether or not a blood transfusion was an adequate procedure.

Transfusion 5. The donor was injected with 4 cc. of gastrin to insure a maximum gastric response. Thirty-five minutes later, stimulation of gastric secretion having become quite manifest, 150 cc. of blood were transfused to a recipient that was secreting free acid and hence more susceptible to gastrin stimulation. The results are shown in table 2, which we interpret as a doubtful negative.

Transfusion 7. The donor was injected with 8 cc. of gastrin in two different areas to insure a more rapid absorption, and with 1 mgm. of histamine to fortify the gastrin. Twenty-five minutes after the gastrin injection and fifteen minutes after the histamine injection, 200 cc. of blood were transfused into the recipient, who was secreting free acid. The results are recorded in table 3. Some increase in the quantity and acidity of the secretin occurred one hour after the transfusion and continued for one and a half hours. Since the latent period of both gastrin and histamine stimulation is never longer than fifteen minutes and since we know that the continuous secretion of a Pavlov pouch dog normally fluctuates some, we are inclined to view this result as doubtfully positive.

GROUP IV. These experiments were performed on a dog with a pouch of the entire stomach and a duodeno-esophageal anastomosis. Obviously such a preparation would be a more delicate test object than a pouch dog (Heidenhain, Bickel or Pavlov).

Transfusion 8. One hour and fifty minutes after feeding the donor, 175 cc. of venous blood were transfused into the recipient, whose gastric glands were secreting free acid (table 4). One hour and ten minutes after the transfusion there was a definite augmentation of secretion. Because another augmentation of secretion occurred again two hours later the result is difficult to interpret.

Transfusion 9. Two hundred cubic centimeters of arterial blood were transfused from a starved (24 hour) gastric fistula dog, that was secreting free acid when the blood was taken, to a recipient with a pouch of the entire stomach that was secreting no free acid. One hour after the transfusion free acid appeared in the secretion of the recipient with an increase in total acid. The result of this experiment indicates that the gastric glands were stimulated by the transfused blood (table 5).

This is the only instance in which glands that were not secreting free acid at the time of the transfusion were stimulated. The result is complicated, however, by the fact that the rate of the continuous secretion of the donor was gradually increasing for some unknown reason prior to the taking of the blood.

TABLE 3

Pavlov pouch dogs

DONOR					RECIPIENT				
Procedure	Time	Gastric secretion			Procedure	Time	Gastric secretion		
		Amount	Free acid	Total acid			Amount	Free acid	Total acid
Transfusion 7									
	o'clock	*cc.*				*o'clock*	*cc.*		
Continuous	10–10:30	0.5	0	0.018	Continuous	10–10:30	3.5	0.319	0.382
	10:30–11	0.9	0	0.027		10:30–11	2.6	0.237	0.319
8 cc. gastrin subcutaneously at 11:05; 1 mgm. histamine at 11:15	11–11:30	15.0	0.255	0.309		11–11:30	2.6	0.237	0.328
200 cc. blood transfused. Took blood 11:15–11:20. Finished 11:25									
	11:30–12	45.0	0.346	0.382		11:30–12	1.6	0.218	0.319
	12–12:30	55.0	0.391	0.419		12–12:30	1.5	0.155	0.246
	12:30–1	41.0	0.455	0.483		12:30–1	3.2	0.237	0.300
	1–1:30	27.0	0.455	0.492		1–1:30	3.3	0.328	0.391
	1:30–2	11.0	0.419	9.455		1:30–2	3.5	0.328	0.401
	2–2:30	3.0	0.391	0.419		2–2:30	1.5	0.255	0.346
	2:30–3	5.4	0.419	0.446		2:30–3	2.8	0.155	0.273
	3–3:30	2.0	0.373	0.419		3–3:30	2.3	0.191	0.291
					Meal at 4:00	3:30–4	1.7	0.191	0.282
						4–4:30	2.5	0.282	0.337
						4:30–5	2.4	0.300	0.355
						5–5:30	4.3	0.373	0.419
						5:30–6	3.5	0.382	0.419

SUMMARY OF TRANSFUSION RESULTS: Only one definitely positive result was observed following the transfusion of "fed" blood (table 1, transfusion 1). All experiments, except one (table 5), in which blood was transfused into dogs secreting no free acid were negative. Of the experiments in which blood was transfused into a dog secreting free acid, one was definitely negative (transfusion 4), two were doubtful (transfusions 5 and 8) and one was doubtfully positive (transfusion 7). The transfused

blood in these cases was either "fed" blood or "gastrin" blood. In the experiment that was definitely positive (transfusion 1) the stimulation was noted during the first hour and it continued at least three hours. In the three doubtful experiments and in the result shown in table 5, it is to be noted that the augmentation of secretion occurred during the second hour after the transfusion.

TABLE 4

GASTRIC FISTULA DOG—DONOR				POUCH OF THE ENTIRE STOMACH NO. 5—RECIPIENT				
Procedure	Time	Gastric secretion		Procedure	Time	Gastric secretion		
			Total acid			Amount	Free acid	Total acid
Transfusion 8								
	o'clock				*o'clock*	*cc.*		
Meal at 1:30					1:30–2	4.4	0.228	0.328
					2–2:30	3.3	0.182	0.255
	2:30	Specimen	0.228		2:30–3	3.2	0.119	0.182
					3 –3:30	2.7	0.110	0.173
175 cc. of venous blood transfused. Took blood 3:12–3:16. Finished 3:20								
					3:30–4	4.5	0.091	0.164
	3:30	Specimen	0.264		4–4:30	3.7	0	0.109
					4:30–5	6.0	0.119	0.210
	4:30	Specimen	0.282		5–5:30	3.2	0.073	0.173
					5:30–6	3.2	0.036	0.118
					6–6:30	4.2	0.045	0.145
					6:30–7	7.5	0.045	0.164
					7–7:30	3.4	0.118	0.200
					7:30–8	3.3	0.073	0.200
					8–8:30	4.0	0.045	0.145
					8:30–9	2.0	0.045	0.164
					9–9:30	1.5	0	0.082
					9:30–10	3.8	0.036	0.109
					10–10:30	4.0	0.109	0.182
					10:30–11	2.8	0.054	0.136
					11–11:30	1.5	0	0.091

The secretory response to a meal is apparently increased following a blood transfusion provided the meal is given within one hour and a half (transfusion 5, table 2).

DISCUSSION OF THE BLOOD TRANSFUSION RESULTS: In experiments involving blood transfusion an effect due to an increase in blood volume per se must be considered. Since the one positive and three doubtful results were obtained in experiments in which the gastric glands were

already forming some gastric juice, it is quite likely that the augmentation in secretion might be due to an increase in blood volume. In only one instance (table 5) did a blood transfusion stimulate glands that were not secreting free acid at the time of the transfusion. It seems, then, that whether or not increased blood volume augments gastric secretion depends on the presence of a preëxisting gastric stimulus. The above exception to this statement (table 5) is not difficult to explain on the basis of the fact that a pouch of the entire stomach was used which is a more delicate test object than a Pavlov pouch. That an increase in blood volume does

TABLE 5

GASTRIC FISTULA DOG—DONOR					POUCH OF THE ENTIRE STOMACH NO. 5—RECIPIENT				
Procedure	Time	Gastric secretion			Procedure	Time	Gastric secretion		
		Amount	Free acid	Total acid			Amount	Free acid	Total acid
Transfusion 9									
	o'clock	*cc.*				*o'clock*	*cc.*		
Continuous	7–8	2.1	0.045	0.155		7–8	2.2	0	0.054
	8–9	4.4	0.182	0.273		8–9	4.4	0	0.036
	9–10	5.2	0.228	0.300		9–10	6.0	0	0.046
200 cc. of arterial blood transfused at 10:15. Taken at 10:12									
	10–11	No collections made				10–11	7.0	0	0.073
	11–12					11–11:30	2.2	0.073	0.164
						11:30–12	1.9	0.045	0.118
						12–12:30	4.5	0.037	0.091
						12:30–1	3.0	0.018	0.082
						1–1:30	4.3	0.073	0.136
						1:30–2	7.5	0.082	0.109
						2:30–3	2.2	0.063	0.136
						3–3:30	2.2	0.045	0.118
						3:30–4	3.1	0.082	0.136

not always stimulate glands that are secreting free acid is readily explained, if we may be permitted to assume that an increase in blood volume does not always cause an increase in the active or minute-volume circulation of the stomach.

If gastrin represents the internal secretion of the stomach or intestine, it is apparently not in the blood in sufficient quantities at any one time to excite the gastric glands. The doubtful, or possibly negative, results that occurred on the transfusion of blood of animals injected with gastrin and histamine suggests that these substances are not in sufficient concentration in the blood at any moment to excite the gastric glands and

that they act as subminimal stimuli, stimulating on accumulation, which falls in line with the observations of Lim and Ammon (1923) and Ivy and Javois (1925). However, it must be kept in mind that in our transfusions we were taking only from one-third to one-fourth of the total blood of the one animal and diluting it to the same amount in the other animal. In other words, we were taking from one-third to one-fourth of the possible circulating secretagogue and diluting it three or four times, which means that for any given brief period of time, the gastric glands of the recipient would be exposed to only from one-ninth to one-sixteenth as much secretagogue as those of the donor. This difference would be quite a material one if we were dealing with a substance that never reaches a very high concentration in the blood.

Our results are not conclusive and convince us that blood transfusion, even under ideal conditions, is an inadequate procedure with which to put to test the possibility of either a hormone or humoral factor in the genesis of gastric secretion.

The nature of our experiments has made it possible for us to report on the effect of hemorrhage on gastric secretion. The results tabulated in tables 2 and 3 show that hemorrhage (the loss of from $\frac{1}{3}$ to $\frac{1}{4}$ of the total blood volume) has no effect on the action of gastrin. The loss of blood by the donor in most instances caused no change in their response to a meal. The donor of transfusion 2 failed to respond to a meal given after the loss of blood and the donors of transfusions 1 and 6 showed a depression in their response to a meal. These observations suggest that hemorrhage does not have much effect on the gastric secretory response after the mechanism has been excited, but that if the hemorrhage precedes the excitation, the mechanism is excited with greater difficulty. We do not have a sufficient number of experiments at this time to warrant such a conclusion, however.

B. Acute cross-circulation experiments. Since we could not find in the literature how much "cross-effect" a drug or some injected substance has on the uninjected animal in a cross-circulation experiment, we found it necessary to perform such experiments. Without consideration of such a "cross-effect" or the amount of circulating substance getting across, it would be more difficult for us to interpret our results.

Methods: Two dogs under ether anesthesia were connected by an arterial-arterial cross-circulation, using special cannulae connected with tubing that could be coated with paraffin and, when clotting occurred, cleaned or replaced in a very short period of time. Such cannulae could be used from 25 to 30 minutes without clotting. Drugs were then injected into one animal and the effect on the two animals compared by means of simultaneous blood pressure tracings and the salivary response when pilocarpine was injected.

Results: Five acute cross-circulation experiments were done, two of them being arterial-venous and three arterial-arterial. Adrenaline (2 cc. of 1:10,000), histamine (1 mgm.) and pilocarpine (3 mgm. subcutaneously) were the drugs used. The amount of "cross-effect" was approximately the same in both kinds of anastomosis.

The relative changes in blood pressure that occurred when adrenalin and histamine were injected are shown in figures 1, 2 and 3. The animal that was injected with the drug always showed the greatest change. In the case of adrenaline the uninjected animal always manifested a fall in blood pressure before a rise, showing therefore the effects of a small and large dose of adrenalin. When 0.5 mgm. of histamine was injected, the blood pressure of the uninjected animals fell only from one-fourth to

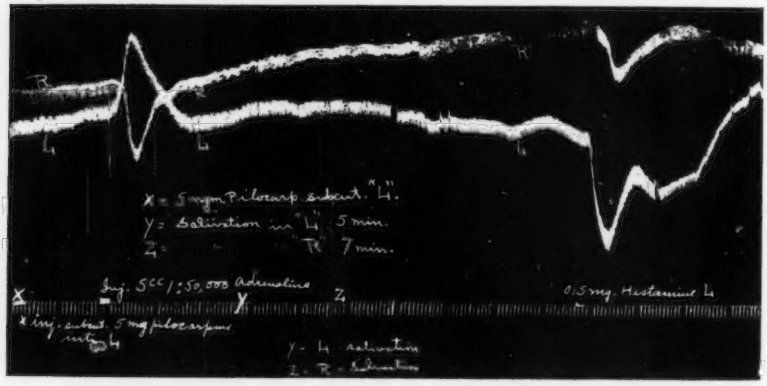

Fig. 1. Tracing shows the effect of adrenalin and histamine on blood pressure in the two animals subjected to an acute cross-circulation experiment. The tracing also shows the relative times that the two animals responded by salivation following an injection of pilocarpine. *L*, left dog; *R*, right dog.

one-third as much as the injected animal. When a larger dose was injected (1 mgm.), the fall of blood pressure in the uninjected animal was from two-thirds to nine-tenths of that of the fall in blood pressure of the injected animal.

Our results on salivation (Wharton's duct cannulated) varied quite widely when pilocarpine was injected (3 experiments). In one experiment the injected animal began salivating in 5 minutes, the uninjected animal in 7 minutes. In a second experiment both animals began salivating at approximately the same time. In a third experiment the uninjected animal began salivating in 6 minutes, the injected animal in 9 minutes. In this last experiment the uninjected animal was showing a definite continuous secretion of saliva at the time the injection was made

into the other animal. These experiments point quite definitely to the important rôle that glandular tonus or reactivity plays in the process of secretion.

SUMMARY AND DISCUSSION OF RESULTS ON ACUTE CROSS-CIRCULATION: It is seen from our results that the amount of cross-effect depends on the

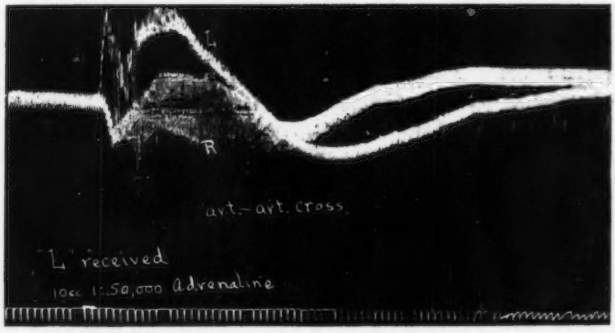

Fig. 2. Tracing from a similar preparation to that used in figure 1. Larger dose of adrenalin used.

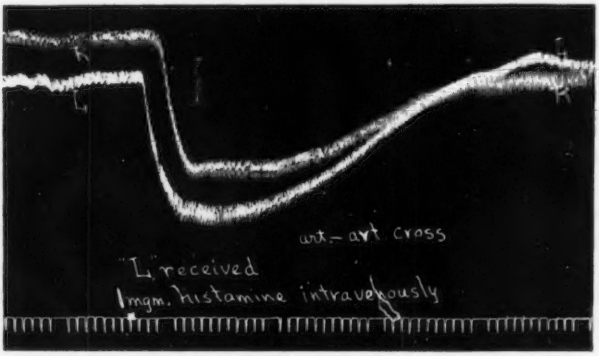

Fig. 3. Tracing from a similar preparation to that used in previous figures. Larger dose of histamine used.

dose of the drug injected. If the dose is large, the cross-effect is from 75 to 90 per cent; if the dose is moderate, the cross-effect is from 25 to 40 per cent. We did not use a small dose, but it is reasonable to believe that if a small dose is used, the cross-effect would be nil or very slight. In the case of the salivary gland, the cross-effect depends to a great extent on the tonus of the gland at the time of injection. If the gland of the unin-

jected animal is secreting at the time of injection, it may respond at the same time or even before the gland of the injected animal.

The amount of the cross-effect of a circulating substance obviously depends on factors other than dose and glandular tonus. The stability of the substance in the blood, the rate of elimination, the rate at which the substance is taken up, combined with and destroyed by the tissues, and the physiological manner of action of the substance, i.e., whether it acts as a minimal or maximal stimulus or through summation, are factors that must be considered. Obviously if one is dealing with a substance that is not eliminated or taken up or destroyed by the tissues, it would in the course of a short period of time be present in the same concentration in the circulation of both animals. But certainly no one would speculate on such a substance being concerned in the stimulation of the gastric glands. In view of the facts at hand it is reasonable to assume a substance whose concentration gradually increases to a maximum and then after a period decreases to a minimal, subminimal or zero level and which is changed or destroyed in the tissues or eliminated. If this is the case, in view of the above results on acute cross-circulation, it is quite probable that gastrins or circulating gastric secretagogues never reach a maximal or much more than a minimal concentration in the circulation of the unfed animal.

C. Cross-circulation experiments on Pavlov pouch dogs

Methods: Only Pavlov pouch dogs that responded well to a meal were used in these experiments. Complete compatibility of the blood of the animals to be operated was tested by cross-agglutination tests.

In brief, the operation was done as follows: The two dogs, being under morphine-ether anesthesia, were placed on the table side by side. Strict asepsis being observed, a longitudinal incision was made in the mid-ventro-lateral portion of the neck of each animal. The skin of the two dogs was approximated dorsally by a buried suture, a few interrupted sutures being taken dorsally in the muscles and subcutaneous fascia. The adjacent carotids of the dogs were carefully dissected from the vagus nerve and internal jugular vein. The carotid of one dog was cut low in the neck and of the other high in the neck, this procedure being necessary in order to furnish a sufficient length of artery to bridge the gap between the two dogs. The two carotids were then anatomosed by the Carrel-Guthrie technique, there being no abnormal tension on the suture or walls of the artery. A strip of carotid from three to four inches long was removed from a third dog and was used to connect the short ends of the carotid of one dog to the short end of the carotid of the other dog. In the instance that an arterial-venous cross-circulation (carotid to carotid and exterior jugular to exterior jugular) was done instead of the first described arterial-arterial, the vein was isolated and cut high in the neck

of one dog and low in the neck of the other, obviously to provide a sufficient length of blood vessel to make the cross possible. The anastomoses being completed, they were shown to be functioning by pulsation in the case of the artery and by the filling of the vein after stripping and clamping it on the cardiac side of the anastomosis. (In the case of the artery, it was necessary, of course, to clamp the artery peripheral to the anastomosis in order to be certain that pulsations were actually coming through from the heart.) Before closing the wound a few interrupted sutures were taken in the muscles ventrally in an attempt to reduce the amount of "dead space," but without causing pressure on the "crossed" blood vessels. At this time muscles and tissues were further examined and dissected in order to prevent kinking of or pressure on the blood vessels. The skin of the two dogs was now approximated by a buried suture, which closed the wound. The dogs were next turned over carefully and each placed on a metal strip for support while a neck and thorax plaster cast was being applied. After the cast dried the animals were placed in a specially devised hammock which held them in a comfortable position.

A functioning cross-circulation was proved again as the experiment progressed, as will be seen by reviewing the tables and protocols.

The usual apparatus for collecting the secretion was used with success. Each time a dog was fed a towel was placed over the eyes and nose of the other dog in an attempt to prevent psychic secretion.

Results: Brief protocols will be given, including what was found at the site of the anastomosis when the animals were separated.

Cross-circulation 1. March 22, 1923. During the forenoon dogs R and B were joined together by a carotid-to-carotid and external jugular-to-external jugular anastomosis in such a manner that dog R donated arterial blood to and received venous blood from dog B. That evening dog R was fed at 10:00. Table 6 shows that he responded to the meal but this response was 50 per cent less than normal. There occurred in dog B a slight increase in the amount and acidity of the secretion of the pouch. Dog B was fed at 3:00 a.m., March 23rd. He ate an entire test meal and responded normally. No noteworthy change occurred in R's secretion. At 9:30 a.m., 2 mgm. of pilocarpine were injected subcutaneously into dog R. Salivation was observed by 9:45 in dog R, but not in dog B. Pupilo-constriction was observed in both. The dogs were separated about 12:00 noon. Examination of the site of anastomosis revealed small clots insufficient to cause much obstruction.

Cross-circulation 2. January 11, 1924. Carotid-to-carotid and external jugular-to-external jugular anastomosis was performed between dogs M and J, dog M donating arterial blood and receiving venous blood from dog J. Operation was started at 3:00 p.m. and completed with dogs in hammock at 5:00 p.m. Observations were commenced the next

day at 12:00 noon. (See table 7.) Dog M drank 350 cc. of milk at 5:00 p.m., but showed only a slight gastric response. Following this procedure dog J's gastric secretion showed some increase in acidity. At 12 midnight dog J ate 150 grams of meat plus 50 cc. of water, the pouch responding to a normal degree for this quantity of meat. Beginning two hours after J had his meal, an increase in the quantity and acidity of

TABLE 6

Cross circulation no. 1

March 22, 1923, Operation performed during forenoon
March 22, 1923. Observations commenced

TIME	DOG R				DOG B			
	Procedure	Gastric secretion			Procedure	Gastric secretion		
		Amount	Free acid	Total acid		Amount	Free acid	Total acid
o'clock		*cc.*				*cc.*		
9–10	Control	1.0	0.054	0.118	Control	1.0	0.018	0.073
10–11	Meal, 150 grams meat, 100 cc. water —10:00	5.0	0.319	0.373		2.0	0.054	0.173
11–12		5.3	0.355	0.410		2.0	0.091	0.173
12–1	March 23rd	4.7	0.364	0.410		1.5	0.136	0.200
1–2		4.0	0.209	0.273		1.5	0.091	0.182
2–3		2.0	0.218	0.291		1.5	0.091	0.182
3–4		1.5	0.155	0.228	Meal, 250 grams meat, 100 cc. water at 3:00	3.6	0.237	0.291
4–5		0.5	0.054	0.182		5.2	0.282	0.346
5–6		2.0	0.127	0.182		8.1	0.346	0.382
6–7		2.0	0.100	0.200		8.3	0.364	0.391
7–8		2.3	0.136	0.218		8.8	0.364	0.391
8–9		2.4	0.145	0.255		8.4	0.328	0.391
9–10	2 mgm. pilocarpine at 9:30	5.6	0.246	0.364	Pupilo-constriction noted at 9:45	7.8	0.346	0.401
10–11	Salivation was noted at 9:45	2.4	0.164	0.237	Salivation was not observed	6.7	0.337	0.391

M's secretion was observed. At 12:00 noon dog M was injected subcutaneously with 1 mgm. of histamine. A definite response resulted in dog M, but no "crossed" effect was observed in dog J. From 2:00 to 4:00 p.m. dog J only secreted a few drops of mucus. At 4:00 p.m. dog M was injected subcutaneously with 3.5 mgm. of pilocarpine. The mucous membranes of dog J became very pale and before they could be separated dog J died. Autopsy revealed dog J to be bloodless and dog M to be engorged

TABLE 7

Cross-circulation no. 2

January 11, 1924. Operation 3:00–5:00 p.m.
January 12, 1924. Observations commenced

TIME	DOG M RECEIVES VENOUS BLOOD				DOG J RECEIVES ARTERIAL BLOOD			
	Procedure	Gastric secretion			Procedure	Gastric secretion		
		Amount	Free acid	Total acid		Amount	Free acid	Total acid
o'clock p.m.		cc.				cc.		
12–2	Control	0.5	0	0.018	Control	0.6	0.110	0.182
2–3		1.8	0	0.055	Dog drank 200 cc.	1.8	0.128	0.201
3–4		1.5	0	0.055	water at 2 p.m.	1.0	0.055	0.128
4–5		1.6	0	0.037		0.5	0.055	0.128
	Dog fed 350 cc. milk at 5 p.m.							
5–6		1.2	0	0.037		0.6	0.064	0.164
6–7		2.0	0.046	0.109		0.5	0.064	0.182
7–8		2.3	0.119	0.182		0.4	0.091	0.228
8–9		2.6	0.109	0.155		0.8	0.082	0.128
9–10		1.8	0.064	0.119		0.4	0.064	0.201
10–11		1.6	0.037	0.091		0.5	0.055	0.182
11–12	Head covered with towel at 11:45 p.m. and while dog J was feeding	2.2	0.027	0.082		0.6	0.046	0.164
					Dog fed 150 grams meat and 50 cc. water at 12 midnight			
a.m.								
12–1	January 13th	0.9	0	0.055		4.5	0.310	0.355
1–2		1.2	0	0.064		6.0	0.382	0.419
2–3		1.9	0.018	0.109		3.8	0.346	0.374
3–4	A suggestive response	2.3	0.055	0.146		2.2	0.292	0.319
4–5		1.7	0.037	0.128		2.5	0.292	0.319
5–6		1.9	0.037	0.128		3.5	0.274	0.301
6–7		2.0	0.027	0.128		2.0	0.283	0.319
7–8		1.8	0.027	0.128		3.9	0.228	0.274
8–9		1.7	0.027	0.110		4.3	0.292	0.337
9–10		2.0	0.027	0.110		3.0	0.274	0.328
10–11	1 cc. 1/2000 adrenal injected subcutaneously at 11 a.m.	1.6	0.037	0.128		0.8	0.110	0.137
		1.6	0.037	0.119	No effect observed	0.7	0.064	0.091
12–1	1 mgm. histamine injected at 12	8.5	0.246	0.310		0.3	0.027	0.128
1–2		7.4	0.310	0.355		0.2	0	0.073
2–3		2.0	0.118	0.209				
3–4		1.0	0	0.063				

with blood. The interpretation of this result is obvious. The anastomosed artery was free from clots as was the vein at the site of the anastomosis. The vein, however, contained at the point where a clamp had been used during the operation a small clot, which was too small to cause much obstruction. The nature of the anastomosis together with the observed facts precludes embolism as the cause of death. This unfortunate accident, however, proves that the cross-circulation had been functioning.

Cross-circulation 3. February 8, 1924. A carotid-to-carotid and carotid-to-carotid anastomosis was performed between dogs S and C, a strip of carotid from a third dog being transplanted between the short central end of C's carotid and the short peripheral end of S's. Operation was started at 2:30 p.m. and the pair placed in the hammock at 4:45. Observations were commenced the next day at 9:00 a.m. (See table 8.) Dog C was offered food at 11:00, but he took only a few bites. A slight gastric response occurred in C. At 8:00 p.m. dog C was offered food and ingested a fairly good meal, but secreted in the course of six hours only about 70 per cent of the usual amount. During the hour after C ingested the meal, dog S showed a return of free acid to his secretion and three hours later secreted slightly more acid than he did at any previous control period. At 3:00 a.m. dog C was injected subcutaneously with 1 mgm. of histamine to ascertain if dog S might not also show a response. The result was negative. Both animals manifesting evidence of thirst, they were given water at 4:00 a.m. Stimulation of gastric secretion resulted in both animals, but a parallel in response was not manifested by the results. Dog S was fed at 6:00 p.m. (February 10). Although the response for the first hour was quite normal for this animal, the succeeding hours were below normal, the normal response for this animal being shown in the latter part of table 8. Following the ingestion of the meal by dog S the secretion of dog C gradually declined until only quantities of mucus insufficient to titrate were collected. At 12:00 midnight dog S was injected with 2 mgm. of histamine, which caused a marked response, but failed to get across to dog C in sufficient amounts to stimulate. At 2:00 a.m. the animals were given water, the gastric glands of neither being stimulated this time as the animals were not so thirsty. At 5:30 a.m. 2 mgm. of histamine were injected into dog C, who showed a usual response. The secretion of S was unaffected. At 11:00 a.m. potassium iodide (100 mgm.) was injected into dog S and obtained in the gastric secretion of C. At 12:00 noon 5 cc. of a one per cent methylene blue solution was injected intravenously into dog C. Dog C's gastric juice was tinged blue in 40 minutes as was dog S's. At 3:00 p.m. 3 mgm. of histamine were injected into dog C, hoping that with this large dose the concentration in the blood of C would be raised so that a quantity suffi-

TABLE 8

Cross-circulation no. 3

February 8, 1924. Operation 2:30
February 9. Observations commenced

TIME	DOG S				DOG C			
	Procedure	Gastric secretion			Procedure	Gastric secretion		
		Amount	Free acid	Total acid		Amount	Free acid	Total acid
o'clock		*cc.*				*cc.*		
9–10	Control	0.9	0.054	0.118	Control	1.8	0	0.036
10–11		1.6	0.054	0.118		1.9	0	0.036
11–12		1.4	0.082	0.136	Dog offered food at 11:00. Only took a few bites	1.9	0	0.082
12–1		1.6	0.082	0.127		3.3	0.109	0.200
1–2		1.8	0.054	0.109		3.2	0.127	0.255
2–3		1.9	0.054	0.109		2.5	0.127	0.218
3–4		2.0	0.045	0.091		2.8	0.127	0.218
4–5		1.5	0.036	0.082		1.3	0.091	0.182
5–6		1.8	0.027	0.072		1.6	0.063	0.136
6–7		1.3	0	0.073		1.2	0	0.136
7–8		2.0	0	0.073		1.3	0	0.073
8–9		1.8	0.045	0.110	Dog ate 150 grams meat, 200 cc. milk, 200 cc. water at 8:00	1.3	0.018	0.082
9–10		1.2	lost			1.7	0.155	0.237
10–11		1.4	0.118	0.164		2.7	0.273	0.337
11–12		1.9	0.082	0.136		3.8	0.282	0.346
12–1	February 10th	0.7	0	0.073	February 10th	4.8	0.300	0.346
1–2		1.1	0.036	0.118		5.7	0.300	0.346
2–3		0.9	0.036	0.082	1 mgm. histamine subcutaneously at 3:00	3.3	0.282	0.337
3–4		0.8	0.036	0.091		5.8	0.300	0.355
4–5	Water ad lib. at 4:00	1.3	0.082	0.127	Water ad lib. at 4:00	4.7	0.246	0.346
5–7		2.5	0.082	0.191		10.0	0.246	0.300
7–8		0.8	0.091	0.136		4.2	0.246	0.291
8–9		1.6	0.109	0.182		3.5	0.264	0.355
9–10		2.5	0.110	0.209		3.0	0.237	0.300
10–11		3.5	0.200	0.246		2.9	0.237	0.273
11–12		3.1	0.200	0.246		2.3	0.209	0.273
12–1		4.0	0.173	0.246		2.5	0.173	0.246
1–2		2.3	0.100	0.118		3.3	0.182	0.218
2–3		2.5	0.091	0.164		2.3	0.145	0.218
3–4		2.1	0.082	0.145		2.6	0.100	0.182
4–5		2.2	0.110	0.173		2.3	0.073	0.164
5–6		2.3	0.036	0.127		0.8	0.054	0.091

TABLE 8—*Continued*

TIME	DOG S				DOG C			
	Procedure	Gastric secretion			Procedure	Gastric secretion		
		Amount	Free acid	Total acid		Amount	Free acid	Total acid
o'clock		*cc.*				*cc.*		
6–7	Dog ate 200 grams meat, 300 cc. water, 8 grams glucose at 6:00	7.3	0.173	0.237		0.6		0.054
7–8		4.1	0.191	0.282		0.4	0	0.027
8–9		1.0	0.182	0.273		0.4	0	0.027
9–10		1.0	0.027	0.118		0	0	0
10–11		1.4	0.027	0.110		0	0	0
11–12	February 10th	1.5	0	0.045		0	0	0
12–1	February 11th 2 mgm. histamine at 12	13.0	0.319	0.282	Mucus insufficient to titrate	0	0	0
1–2		16.3	0.373	0.455		0	0	0
2–3	130 cc. water at 2:00	0.7	0.182	0.255	195 cc. water at 2:00	0	0	0
3–4		1.0	0	0.127		0	0	0
4–5		1.0	0	0.063		0	0	0
5–6		1.0	0	0.054	2 mgm. histamine at 5:30	2	0	0.091
6–7		1.4	0	0.054		8.5	0.319	0.364
7–8		2.5	0	0.063		2.6	0.309	0.364
8–10		1.6	0	0.073		0.8	0.209	0.282
10–11		1.5	0	0.054		1.3	0.127	0.200
11–12	Inj. 100 mgm. KI at 11:00	2.1	0.036	0.091	KI in gastric juice	0.8	0.045	0.100
12–1	Methylene blue in juice at 12:45	3.0	0.110	0.164	Injected methylene blue at 12:00	2.3	0.054	0.118
1–2		1.4	0.100	0.164		1.7	—	—
2–3		3.2	0.045	0.127		2.5	0.136	0.209
3–4	Histamine got through?	2.3	0.110	0.218	3 mgm. histamine at 3:00	7.0	0.337	0.382
4–5		1.3	0.073	0.164		1.9	0.337	0.401
5–7	2 hours	3.5	0.054	0.136		1.6	0.110	0.218
7–8		1.0	0.063	0.145		1.0	0.054	0.164
8–9		2.0	0.091	0.182	Meal 100 grams meat, 200 cc. milk, 100 cc. water at 8:00	5.2	0.291	0.345
9–10		1.6	0.091	0.182		3.0	0.328	0.401
10–11		2.0	0.110	0.200		5.1	0.346	0.382
11–12		1.8	0.118	0.200		4.6	0.337	0.382
12–1	February 12th	2.0	0.164	0.237		3.8	0.328	0.373
1–2	The most suggestive response	2.0	0.164	0.237		2.2	0.319	0.364
2–3		2.5	0.136	0.182		3.7	0.328	0.373
3–4		2.0	0.164	0.237		2.1	0.328	0.373

TABLE 8—*Concluded*

TIME	DOG S				DOG C			
	Procedure	Gastric secretion			Procedure	Gastric secretion		
		Amount	Free acid	Total acid		Amount	Free acid	Total acid
o'clock		cc.				cc.		
4–5		1.3	0.136	0.218		3.3	0.319	0.364
5–6		1.2	0.054	0.155		1.9	0.309	0.364
6–7	Meal 230 cc. milk, 200 grams meat at 6:00	2.1	0.082	0.155		1.9	0.182	0.273
7–8		7.5	0.291	0.346		2.5	0.273	0.319
8–9	Methylene blue injection at 8:40	15.0	0.346	0.401		2.0	0.300	0.355
9–10		12.6	0.401	0.446	Methylene blue in gastric juice	1.2	0.246	0.291
10–11		7.5	0.346	0.391		1.6	0.228	0.291
11–12		8.0	0.337	0.382		2.5	0.255	0.328
12–1		7.0	0.346	0.401		1.2	0.246	0.328
1–2		10.1	0.346	0.410		1.7	0.246	0.328

cient to stimulate would cross over to dog S. The result suggested that a small quantity passed through. Dog C was fed at 8:00 p.m. and it is to be noted that the gastric response is better than at the previous feeding, although a smaller quantity of meat was eaten. Following this meal eaten by dog C, dog S showed the most suggestive response (table 8) that we have observed, dog S not having received any food for 26 hours. At 6:00 a.m. February 12, dog S ingested quite a large meal to which he responded normally. Dog C showed no material change in gastric secretion. Methylene blue injected into dog S at 8:40, appeared in his gastric juice at 8:50 and in the gastric juice of dog C at 9:00.

The dogs were put under an anesthetic at 3:00 p.m. in order to separate them. An examination of the anastomoses showed the transplant to be patent with only a film of clot at the site of the anastomosis and the direct anastomosis or non-transplanted cross to be quite patent, but slightly more clot was present at the site of this anastomosis and a small dissecting aneurysm had developed. These facts together with our results on methylene blue prove that the cross-circulation had been functioning.

A fourth experiment was attempted, but was unsuccessful.

SUMMARY OF RESULTS ON CROSS-CIRCULATION IN PAVLOV POUCH DOGS: It is manifest from our results that a long time cross-circulation can be performed with success in dogs because the functioning of the cross has

been demonstrated seventy-two hours after the operation and also because of the observations at autopsy.

In the three experiments performed observations were made on the response to eight meals with the result that the response in the unfed dog following a meal to his "twin" might be interpreted as suggestive (expt. 1, dog B; expt. 2, dog M; expt. 3, dog S, February 9th) in three cases and slightly positive but not decisive, in one (expt. 3, dog S, February 11th and 12th, table 8). The suggestive responses occurred from one to three hours after a meal.

When 1 mgm. of histamine, a maximal gastric secretory dose for most dogs, was injected subcutaneously in one dog no cross effect was observed. When 3 mgm. of histamine were injected, a suggestive cross effect occurred (dog S, table 8).

DISCUSSION OF CROSS-CIRCULATION EXPERIMENTS ON PAVLOV POUCH DOGS: The three suggestive results cited in the above summary that occurred can readily be accounted for by variations in the continuous secretion of the pouch. In spite of the fact that such a coincidental variation occurred in three instances we are inclined to interpret them as coincidental because of the absence of a cross response following four other meals. The much more suggestive response that occurred in dog S, experiment 3 (table 8), is more difficult to explain on the basis of a variation in continuous secretion because, if it is, it is quite an exceptional one. Obviously, it is not a decisive result. We feel that only a decisive result would warrant the conclusion that there is something circulating in the blood following a meal that stimulates the gastric glands.

The fact that the suggestive responses occurred from one to three hours after the meal was fed rules out the possibility that the response might be due to psychic secretion in the unfed dog.

Since we could inject 3 mgm. of histamine, a supermaximal dose, and only observe a suggestive cross effect on gastric secretion, we believe that less than one-sixth of the circulating secretagogue, if such exists, was getting across to the gastric glands of the unfed dog. If this is the case, one should possibly not expect more than a minimal response, if any. Because of this fact and the possibilities and facts presented in our previous discussions of this matter, we believe that a cross-circulation experiment on Pavlov pouch dogs is not an experimentum crucis, if negative or indecisive results are observed.

GENERAL DISCUSSION

We have attempted to solve the question of a humoral mechanism in the genesis of gastric secretion under conditions and by methods that certainly must be considered on first thought as ideal. However, the

results which have been to a certain extent suggestive have not been decisive. We feel that only decisive results should warrant a definite conclusion. We have suggested in our discussions possible factors based on facts that might invalidate our results and that might explain their negative or indecisive nature, such as the condition of the gland cells at the time of the experiment, the normal variations in the continuous secretion, the subminimal manner by which gastrin and histamine stimulate and the possibility of a low concentration in the blood of the alleged circulating secretagogue. Because of these considerations, in view of our results, the method used might be considered as inadequate for the purpose of solving the question at hand.

However, results that will be presented in a following communication on the secretion of a completely transplanted gastric pouch (subcutaneous into the mammary gland) suggest that the results obtained in our cross-circulation experiments on Pavlov pouch dogs should possibly be interpreted as positive and that we have been ultracritical with respect to those results.

CONCLUSIONS

A. 1. Blood transfusion, even under the best of conditions, is an inadequate procedure with which to put to test the possibility of either a hormone of a humoral factor in the genesis of gastric secretion, because it is apparent from our results that there is an insufficient amount of exciting substance, if such exists, in the transfused blood at any moment to cause a definite gastric response.

2. It is suggested that substances that excite gastric secretion on hypodermic injection stimulate by cumulative action.

3. An increase in blood volume enhances the gastric secretory response to a meal, provided the meal is given shortly ($\frac{1}{2}$ to 1 hour) after the blood transfusion. An increase in total blood volume may augment gastric secretion, provided the glands are acting at the time of transfusion.

4. The effect of hemorrhage on gastric secretion is discussed.

B. 1. It is quite apparent from our results that the cross-effect of an hemodynamic drug depends to a great extent on the dose, the larger the dose the greater the cross effect and vice versa. In the case of a secretory drug, the cross effect depends chiefly on the condition of the gland at the time the drug is used. If the gland is showing a continuous secretion at the time of injection, the latent period of the cross effect is shortened.

2. Other factors that have a bearing on our problem and that might explain negative or only slightly positive results occurring in cross circulation experiments to determine a humoral mechanism for gastric secreton are discussed.

C. 1. Long time (72 hours) cross-circulation experiments can be performed with success on dogs.

2. On feeding a meal to one of a pair of cross-circulated Pavlov pouch dogs, a result suggestive of stimulation of the gastric glands of the unfed dog occurred in four out of eight tests. We interpret the results as suggestive, but not decisive, because the variation in secretion that occurred might be accounted for by a coincidental variation of the continuous secretion.

3. On the basis of the results following histamine injection into one of the animals of the pair, the conclusion is warranted that less than one-sixth of the histamine passes across to the uninjected animal.

4. We doubt that a cross circulation is to be considered as an experimentum crucis for the problem under study.

5. Our results possibly suggest, but do not warrant, the conclusion that a humoral mechanism is concerned, in part, in the genesis of gastric secretion.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

VIII. The Proof of a Humoral Mechanism[1]

A NEW PROCEDURE FOR THE STUDY OF GASTRIC PHYSIOLOGY

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Although a humoral mechanism for gastric secretion has been postulated for many years, evidence clearly proving such a mechanism has not been offered.

In a preceding article of this series, Ivy, Lim and McCarthy (1925) have attempted to settle the question by blood transfusion and cross-circulation experiments performed on Pavlov pouch dogs under apparently ideal conditions. Results were obtained that were suggestive, but not decisive, of a humoral mechanism being concerned in gastric secretion. They pointed out that a blood transfusion procedure was inadequate and that a cross-circulation experiment does not necessarily constitute a crucial experiment in solving the question at hand.

In this paper we will present evidence obtained by use of a new procedure for the study of gastric physiology, namely, the subcutaneous transplantation of a gastric pouch.

Method. *The subcutaneous transplantation of a gastric pouch:* Female dogs were selected that had recently whelped or weaned pups in order to assure a maximum vascularity of the mammary gland into which the pouch was to be transplanted.

The operation was done in two stages. *First stage:* A portion of the fundus was selected that was supplied by one artery. This portion was resected and made into a pouch. The resulting pouch in most instances measured from 6 to 8 cm. in length and from 3 to 5 cm. in diameter. An incision was made in the skin at the border of gland and a stab wound incision carefully made without severing large blood vessels. A cavity of sufficient size to accommodate the gastric pouch was made in the mammary gland by spreading the tissues with a hemostat. The gastric pouch was drawn through the stab wound opening and sutured with only a few stitches in the cavity prepared for it.

[1] Preliminary report, This Journal, lxxii, 203, 925.

Second stage: From two to four weeks after the first operation, after healing was complete and observations on the secretion of the pouch had been made, an incision was made lateral to the pouch, care being taken not to sever large blood vessels. Through this incision the epiploic artery and vein that supplied the pouch was tied, severed and peritonealized.

Proof of the transplant: Under an anesthetic the coeliac artery was isolated and injected with a colored solution that is usually used for such purposes. None of the injecting fluid reached the blood vessels of the pouch, showing that the pouch received no blood from the coeliac artery or its branches.

Fig. 1. Picture of a transplanted gastric pouch. This pouch is as mobile as the mammary gland, i.e., the finger can be inserted into the pouch and by this means the pouch can be moved about as freely as the mammary gland.

RESULTS. A gastric pouch has thus been completely transplanted into the mammary gland of five female dogs. Following the second operation some sloughing occurred in two cases. In one of these sloughing occurred to such an extent that the inner half of the pouch granulated and the animal had to be discarded. Adhesions to the peritoneum at the site of the stab wound was found only in one case. These adhesions were severed (table 1).

Continuous secretion: Nothing but a thick mucous secretion of low total acidity (0.018) was observed. The fluctuations in continuous secretion that so frequently occur in the Pavlov and Heidenhain pouch preparations were never observed. In quantity the continuous secretion

varied from an amount that could not be collected by the usual methods to one cubic centimeter per hour.

For the first two or three days after the second operation a fairly copious (2 or 3 cc. per hour) thin, mucoid, continuous secretion of very low total acidity was collected. This secretion was turbid and in two cases contained no blood, in two a trace of blood, in another a large quantity of blood and necrotic mucosa (this last dog was discarded). We believe

TABLE I

Transplanted gastric pouch dog: W

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
Response before severing original blood supply of pouch					
	o'clock	*cc.*			
Continuous	9–12	0.8	0	0.036	
Meal at 1:00	12–2	0.5	0	0.036	
	2–3	2.0	0.191	0.291	
	3–4	1.0	0.155	0.264	
Response of the completely transplanted and isolated pouch					
Continuous	12–3	1.9	0	0.045	
	3–6:30	2.0	0	0.045	
Meal at 6:30	6:30–9:30	2.0	0	0.091	
	9:30–11:00	2.0	0.036	0.127	Latent period about 3 hours
	11:00–12:00	1.0	0.036	0.127	
	12–1	1.0	0.027	0.136	
	1–2	0.9	0	0.045	
	2–4:30	2.0	0	0.027	
Response after exploratory operation. Dr. Carlson assisting. Adhesions found and severed					
Continuous	10–11	0	0	0	
Meal at 11:00	11–1	0.8	0	0.018	
	1–4	1.0	0.110	0.182	
	4–6:30	1.5	0.054	0.110	0.5 cc. of the juice digested 4 mm. by Mett's tube method
	6:30–10:30	0.5	0	0.027	

that this fluid was a transudate due to edema or passive hyperemia of the pouch rather than a "paralytic" secretion because it contained no free acid.

Response to a meal: An increase in volume and total acidity always occurred from two to five hours after a meal. Infrequently an increase in total acidity occurred in the secretion collected the first hour after feeding. Free acidity was observed rather infrequently from two to

six hours after a meal. In one instance the free acidity amounted to 0.127 per cent, in which instance the total acidity was 0.273 per cent. In most instances the free acidity persisted in the secretion from two to three hours, but in one case it was present for twenty hours. In most instances the rise in total acidity persisted about twelve hours, but in one instance it was present for twenty-four hours (see tables 1, 2, 3, 4).

The largest quantity of secretion collected in one hour was 2.3 cc., the average being about 1.5 cc. per hour.

TABLE 2

Transplanted gastric pouch dog: B. W.

PROCEDURE	TIME	GASTRIC SECRETION		
		Amount	Free acid	Total acid.
Response to meal 10 days after second operation				
	o'clock	*cc.*		
Continuous	7:30–9:00	3.0	0	0.009
Meal at 9:00	9:00–10:30	1.5	0	0.063
	10:30–11:30	1.4	0	0.063
	11:30–12:30	1.5	0.082	0.173
	12:30–1:30	1.8	0.091	0.182
	1:30–3:00	2.3	0.091	0.145
	3–4	1.7	0.100	0.145
	4–5	1.4	0.073	0.218
	5–6	1.9	0.082	0.182
	6–7	0.9	0.036	0.110
	7–8	0.8	0.036	0.073
	8–9	1.2	0	0.091
	9–10	0.3	0	0.036
	10–11	0.8	0	0.036
Response to meal 3 months after second operation completing the transplantation				
Continuous	9–11	0.7	0	0.009
Meal at 11:00	11–12	0.8	0	0.018
	2–4	1.5	0.027	0.082
	4–5:30	2.2	0.027	0.082
	5:30–7	2.5	0.063	0.127
	7–11	3.0	0.063	0.118

The secretion of the pouch following a meal contains a normal amount of pepsin (tables 1 and 3).

We have found that the pouch is more likely to be stimulated to secrete a juice containing free acid if the animal is starved for one day and then given all the food he would eat with water ad libitum.

Response to histamine and gastrin[2] *subcutaneously.* In all of the animals

[2] We thank Dr. F. C. Koch for supplying us with gastrin for use in these experiments.

TABLE 3

Transplanted gastric pouch dog: B.

PROCEDURE	TIME	GASTRIC SECRETION			PEPSIN
		Amount	Free acid	Total acid	
Response to meal 2 weeks after first operation					
	o'clock	*cc.*			*mm.*
Continuous	9–12	1.0	0	0.036	
	12–1	2.0	0	0.027	
Meal at 1:00	1–2	4.0	0.027	0.218	
	2–3	8.0	0.155	0.264	
	3–4	6.0	0.155	0.264	
	4–5	4.5	0.045	0.200	
Response to meal 4½ months after second operation in completing the transplantation					
	9–10	0.7	0	0.036	
Meal at 10:00	10–11	1.0	0	0.063	
	11–12	1.0	0.009	0.110	
	12–1	1.2	0.054	0.155	8.0
	1–2	1.3	0.063	0.173	4.0
	2–3	1.2	0.009	0.100	3.5
	3–4:30	1.5	0.054	0.127	
	4:0–6	1.4	0.054	0.127	
	6–7	1.2	0.027	0.118	
	7–9	1.8	0	0.082	
	9–11	1.4	0	0.063	
	11–12:30	1.0	0.009	0.091	
	12:30–2	1.5	0	0.054	

TABLE 4

Transplanted gastric pouch: Ben

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
Response 5 days after second operation completing the transplant					
	o'clock	*cc.*			
Continuous	9–10	0	0	0	
Meal at 10.00	10–11	0.4	0	0.009	Mucous
	11–1	2.8	0.027	0.127	
	1–1:40	1.0	0.063	0.155	
	1:40–2:40	2.1	0.091	0.182	
	2:40–3:40	2.0	0.136	0.237	
	3:40–4:40	1.6	0.164	0.264	
	4:40–5:40	1.8	0.091	0.246	
	5:40–6:40	1.2	0.063	0.228	
	6:40–7:40	1.1	0.054	0.218	
	7:40–8:40	1.8	0	0.054	Mucous

an increase in total acidity was observed following the subcutaneous injection of 1 mgm. of histamine. Only one of them (B. W.) secreted a

TABLE 5

Showing the response of subcutaneously transplanted gastric pouches to the subcutaneous administration of histamine and gastrin

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
Transplanted gastric pouch dog W.					
	o'clock	*cc.*			
Continuous	11–12	0.7	0	0.009	
1 mgm. histamine at 12	12–1	0.7	0	0.027	
	1–2	1.0	0	0.036	
	2–3	0.9	0	0.036	
3 mgm. histamine at 3:00	3–4:30	1.0	0	0.054	
	4:30–6	1.0	0.054	0.164	
	6–7	1.0	0.018	0.110	
Transplanted gastric pouch dog B. W.					
Continuous	11–12	0.3	0	0.009	
1 mgm. histamine at 12	12–1	1.4	0.110	0.145	Shortest latent period observed
	1–2	0.5	0.127	0.191	
	2–3	1.0	0	0.045	
	3–4	0.7	0	0.036	
	4–5	0.7	0	0.018	
Transplant gastric pouch dog B.					
Continuous	10–11	0.5	0	0.018	
Continuous	11–12	0.5	0	0.018	
1 mgm. histamine at 12	12–1	1.0	0	0.027	
	1–3	1.2	0	0.045	
3 mgm. histamine at 3:00	3–4	2.0	0	0.045	
	4–5	1.3	0.091	0.228	
	5–6	1.0	0	0.091	
	6–11	2.0	0	0.027	
Transplant gastric pouch dog B. W.					
Continuous	2–4	0.5	0	0.009	
4 cc. of Koch's gastrin at 4:00	4–5	0.6	0	0.054	
	5–6	2.0	0.182	0.228	
	6–7	1.0	0.155	0.228	
	7–8	0.7	0	0.045	

juice showing free acid when the above dose was administered. All responded by secreting a juice containing free acid when from 2.5 to 3 mgm. of histamine were injected. The latent period of response was

longer than is observed in a Pavlov or Heidenhain pouch. Sometimes it was as long as an hour, the latent period of the Heidenhain pouch being approximately 15 minutes (table 5).

The largest quantity of secretion collected in one hour following histamine was 3.0 cc. The highest acidity observed was 0.228 per cent free acid and 0.273 per cent total acid (B. W.), which is also the highest acidity recorded following a meal.

Four cubic centimeters of gastrin subcutaneously caused usually after a latent period of one hour a response in the transplanted pouch. One or 2 cc. only caused an increase in total acidity (table 5).

The quantity and quality of the secretion. It will be noted from the tables that the acidity was increased relatively to a greater extent than the quantity when the glands of the pouch were stimulated by a meal or histamine. The peptic contents of the secretion of the pouch was normal in concentration, but low in total quantity.

The importance of blood volume flow. Following the second operation, at which the original gastro-epiploic blood supply of the pouch was severed, there occurred in every instance a decrease in the response of the pouch to a meal. This is demonstrated very definitely in the results recorded in table 3 in which dog the decrease was most marked. This decrease in total blood supply of the pouch affected the quantity of the secretion to a greater extent than the quality, although both were diminished. It was more difficult after the second operation to stimulate the pouch to secrete free acid with a meal or with histamine than before. Before the second operation the pouch responded like a Heidenhain pouch, which it was except for a slight modification. These observations certainly demonstrate the importance of a normal or adequate blood supply for gastric secretion.

Attempts to excite the pouch by other procedures. Two hundred milligrams of histamine were given by mouth (Ivy and Javois, 1925) with 100 cc. of water with negative results. Three hundred cubic centimeters of N/10 HCl were given by mouth also with negative results.

Since the transplanted gastric pouch is quite refractory, being stimulated only by maximum stimuli (a large meal and a maximum dose of gastrin and histamine), no other attempts were made to excite its glands by other substances. We believe that such attempts would only be attended by negative results.

Distention of the transplanted pouch. During our studies on the motility of the transplanted pouch during hunger we had ample opportunity to study the effect of distention of the pouch on its secretion. In one instance out of some twenty trials, we found that the glands of the pouch were stimulated to secrete free acid.

This observation is quite significant in view of the fact that throughout all of our work on the continuous secretion of the pouch we failed to ever observe the presence of free acid.

Effect of estrus, pregnancy and lactation. We have been able to follow one of our dogs (B) through heat, pregnancy and lactation, periods during which the blood supply of the mammary gland is increased. We found that the transplanted pouch following a meal secreted approximately

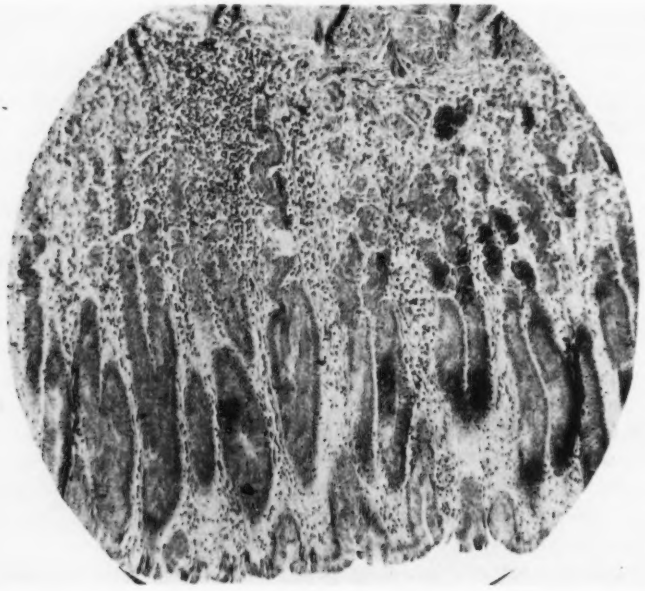

Fig. 2. Low power section through the mucosa of a pouch that had been transplanted seven months. Note atrophy, infiltration of round cells and connective tissue, and decrease in number of chief and parietal cells. Atrophy is not marked, however.

25 per cent more juice than before pregnancy, but the acidity of the juice and time of appearance was unaffected.

This result shows that the increased blood flow brought about by the hyperplasia of the mammary gland affected the quantity rather than the acidity of the secretion of the transplanted pouch.

Histology[3] *of the transplanted pouch.* Up to the present time we can only report on the histology of one of the transplanted pouches. It had been transplanted about seven months.

[3] We desire to express our thanks to Dr. A. B. Dawson who made the histological examinations for us.

The mucosa manifests definite signs of atrophy. There is an increase in connective tissue throughout and in some areas there are collections of small round cells. There is a decrease in the number of parietal and chief cells and in places the base of the glands is disorganized by connective tissue (fig. 2).

Grossly the mucosa appeared to be normal. Physiologically our evidence shows that the atrophy either is not progressive or is not progressing at a very rapid rate. We believe the atrophy that is present is due to the immediate injury caused at the second operation at which the original blood supply is cut off from the pouch.

Discussion. Although our results show quite decisively that a humoral mechanism is a factor that is concerned in the genesis of gastric secretion following the ingestion of a meal, it does not follow that the causative agent is a hormone. There are four hypothetical possibilities that occur to us that might be causal agents concerned in this humoral mechanism of secretion of the pouch: *a*, a hormone; *b*, secretagogues present in or resulting from the digestion of the food; *c*, a "digestive blood state," by which we mean that some change occurs in the blood due to the secretion of the digestive glands, such as a change in reaction, a mobilization of substances necessary for secretion, etc., which renders the blood favorable for the separation of gastric juice by the gastric glands of the pouch; and *d*, an increase in the blood flow to the mammary gland, therefore to the transplanted pouch, incident to circulatory changes that might possibly occur during the digestion and absorption of a meal.

The suggestive results observed by Ivy, Lim and McCarthy (1925) in their cross-circulation experiments argue against the last mentioned hypothesis as does also the fact that the transplanted pouches continued to secrete to a meal four to eight months after post-lactation atrophy of the mammary gland. Also, in view of our observation that estrus, pregnancy and lactation only lead to an increase in the quantity and not the quality of the secretion the latter hypothesis is hardly tenable.

We know of no evidence bearing directly on the third hypothesis. There is ample evidence, however, that either one or both of the first two hypotheses might be concerned and we personally believe that either one or both are involved in the humoral mechanism of gastric secretion.

It might appear as if the long latent period, which occurs before stimulation of the glands of the pouch is manifest following a meal would argue against a hormone and for secretagogues as being the exciting agent. If a hormone were the exciting agent, one should only expect a latent period of from ten to twenty minutes. Whereas, if secretagogues yielded by digestion were the exciting agent, we would predict a long latent period of an hour or more, as was actually found. This argument will not hold, however, because the transplanted pouch manifests an abnor-

mally long latent period when histamine and gastrin are injected. The long latent period, then, is only an "abnormal characteristic" of the transplanted pouch associated with its observed refractoriness. Hence, it is impossible for us to conclude or even express a worthwhile opinion as to whether the humoral agent is a hormone or secretagogues.

The long latent period and refractoriness which the transplanted pouch manifests can be explained in three ways. *a.* There is a definite change in the histology of the mucosa. There is an increase in connective tissue and a decrease in parietal and chief cells. Increase in connective tissue is usually interpreted to mean a decrease in blood supply, which associated with an actual decrease in the number of secreting cells would quite certainly make the gland more refractory. *b.* The glands of the pouch may be more refractory because of the complete section of its extrinsic nerves and reduced blood supply. It is well known that the partially denervated Heidenhain pouch is more refractory than the Pavlov pouch—only the vagi are cut in the Heidenhain pouch. The transplanted pouch is completely denervated and its blood supply is less than normal. The response of a transplanted pouch to a meal, histamine and gastrin is quite like the response of a partially atropinized Pavlov pouch. It is quite likely that the extrinsic nerves play a rôle of maintaining the glandular threshold at a low level, so that the normally innervated gland responds to a weaker stimulus than an extrinsically denervated gland. It appears, therefore, that the transplanted pouch may be somewhat refractory because of complete section of its extrinsic nerves and a reduction of its normal blood supply, irrespective of the histological change it manifests. *c.* The transplanted pouch may be more refractory than the Pavlov or Heidenhain pouch because the blood vessels of the mammary gland and of the pouch (after nerve section) respond to a less degree and more slowly to histamine, gastrin or circirculating secretagogues than do the normally innervated splanchnic blood vessels.

It should be noted that three observations have been made which have some bearing on the factors concerned in the quality and quantity production of gastric juice. *a.* As compared with other gastric pouches, a meal causes a greater increase in the acidity than in the volume of the secretion of the transplanted pouch. *b.* Severing the original blood supply at the second operation has a greater effect on the quantity than on the acidity of the secretion. *c.* An increase in blood flow to the mammary gland increases the quantity, but not the acidity of the secretion obtained after a meal. These observations seem to indicate that the blood volume flow is more intimately related to volume production, whereas the humoral agent is more intimately related to acid production.

Such a conclusion is not warranted, however, because the situation is complicated by the histological condition of the pouch.

The fact that the glands of the transplanted pouch were stimulated by mechanical distention shows that the mechanism concerned in this type of stimulation is at least partly local, which is a confirmation of the position taken by Lim, Ivy and McCarthy (1925) in a previous article on this subject.

SUMMARY AND CONCLUSIONS

1. A gastric pouch can be transplanted subcutaneously by a suitable but simple technique.

2. The transplanted pouch does not show appreciable fluctuations in the continuous secretion. This secretion never contains free acid and only a small amount of total acid.

3. Such a pouch secretes a gastric juice containing acid after a meal and after histamine and gastrin administered subcutaneously. The latent period of response after a meal is from two to six hours. Following histamine or gastrin the latent period is usually one hour.

4. The transplanted pouch is more refractory than a Heidenhain pouch. The possible factors that might be involved in the causation of this refractoriness are discussed.

5. In our experiment we were successful in stimulating the glands of the pouch by mechanically distending it with a balloon, which proves that a local mechanism is concerned, at least in part, in this type of stimulation.

6. The evidence presented shows decisively that a humoral mechanism is one of the mechanisms concerned in the genesis of gastric secretion.

Our evidence does not prove or disprove a hormone hypothesis. Various possible factors that might be involved in the humoral mechanism of secretion of the pouch are mentioned and discussed.

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THE EFFECT OF AUTO-CONDENSATION AND HIGH FREQUENCY ELECTRIC CURRENT ON THE TONUS OF SKELETAL MUSCLE

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The treatment of disease by electric current of various kinds is widely practiced at the present time and, in general, the results are well known. Athletic trainers are using auto-condensation and high frequency currents quite extensively in treating tired, and in some cases, normal skeletal muscle.

Although the experience of the subjects indicates that benefit is derived from electric treatment, there are no data, as far as the writers have been able to find, which show the variation and extent of such treatments. In this investigation, normal skeletal muscle was subjected to both auto-condensation and high frequency currents. The activity of the muscle before and after the treatment was recorded and compared.

It has been shown by Lombard (1887), Dodge (1911), Mitchell and Lewis (1886), Brown (1925), Emery (1925) and Tuttle (1924) that whatever increased or decreased the body tonus increases or decreases the extent of the knee-jerk accordingly. It is thus reasonable to suppose that if electric treatments affect the tonus of skeletal muscle the result of such disturbances will be manifest in the extent of the knee-jerk. In order to determine the effect of auto-condensation and high frequency electric currents on skeletal muscle, the extent of the knee-jerk before and after treatment was compared.

The knee-jerk was elicited and recorded by means of an apparatus described by Tuttle (1924a). The machine is so constructed that it delivers uniform stimuli of equal intensity at regular intervals to the ligamentum patellae. The forward component of the excursion of the foot is used as the index of the activity of the muscles of the thigh and is recorded by a stylus as a vertical mark on a smoked drum. The stimuli in this investigation were delivered to the center of the ligamentum patellae at the rate of seven per minute. The intensity of the stimuli was varied according to the strength necessary to elicit a response well below the maximal. The records read from right to left due to the fact that the baseline is at the top when they are made.

The electric current used was delivered by the Fisher (type G) Diathermy Outfit. This machine is capable of delivering both auto-condensation and high frequency currents. It is movable and especially adapted for experimental purposes.

THE D'ARSONVAL AUTO-CONDENSATION CURRENT. In this experiment nine different subjects were used. They were all untrained in knee-jerk experiments and had had no experience with auto-condensation current.

In place of the pad usually employed, one was especially constructed with a hinged back so that it would fit in the bottom and back of the barber's chair. The subjects removed sufficient clothing so that there was direct bodily contact with the pad from the knees well up the back. A hollow metal rod grasped in both hands served as an electrode to complete the circuit.

The subjects were seated in the apparatus and instructed to close the eyes and remain as passive as possible. While the records were being made the subjects were left alone so that distracting influences were reduced to a minimum. In all cases the high voltage terminal was used so that the desired strength of current could be obtained with as short a spark gap as possible. This plan was adopted since it was found that in some cases a noisy spark was distracting to the subject.

The general method of procedure was to record a sufficient number of jerks to obtain a record characteristic of the subject at the time of the experiment. As soon as this was completed and without notice to the subject the current was turned on for thirty minutes. The experiment was begun with from 100 to 200 milliamperes. The strength of the current was gradually increased to a maximum of 750 milliamperes. At the end of thirty minutes the machine was started and the extent of the knee-jerk recorded. This technique allowed the subject to remain in the apparatus and thus eliminate the possibility of variation in adjustments. A study of the latency of the effect of the current was abandoned due to the fact that a spark occurred between the knee and the stimulating hammer which was sufficiently uncomfortable to ruin the experiment.

The results obtained are shown in table 1 in which a comparison of the activity of the knee-jerk mechanism before and after auto-condensation treatment is made. The per cent of variation in activity is also given.

Two pairs of records are given in figures 1 and 2 which show the effect of auto-condensation current on the knee-jerk.

In all cases except subject 9, the extent of the knee-jerk was reduced by the auto-condensation current. The range of decrease was from 16 per cent to 96 per cent. Subject 9 showed an increase due to excitement which was plainly visible. During the first trial subject 3a showed an increase in the knee-jerk after the auto-condensation treatment. However, during the second trial she showed a material decrease. Both

observations and report supported the judgment of the writers that during the first trial the subject was excited but was not during trial two.

Subject 4 did not appear to be responding to the electric current. After a treatment of 750 milliamperes for thirty minutes, a short record was

TABLE I

Comparison of the height of the knee-jerk of nine subjects before and after they had been subject to D'Arsonval auto-condensation current

SUBJECT	TOTAL HEIGHT OF KICKS	NUMBER OF KICKS	AVERAGE HEIGHT OF KICKS		PER CENT OF DIFFERENCE
			Before	After	
	mm.		*mm.*	*mm.*	
1	271	52	5.27		
	95	36		2.64	49
2	129	37	3.49		
	6	44		0.136	96
3	3,798	56	67.82		
	2,844	54		52.66	22
3a	371	42	8.88		
	344	36		9.55	7 (increase)
4	1,132	40	28.30		
	1,253	53		23.64	16
	780	79		9.87	65
5	1,666	49	34.00		
	615	42		12.55	63
6	949	49	19.36		
	403	43		9.37	51
7	448	24	18.66		
	65	40		1.62	91
8	123	36	3.41		
	69	54		1.27	62
9	141	44	3.20		
	75	21		3.57	11 (increase)

made. A second treatment was decided upon with a strength of 1000 milliamperes. In about ten minutes the subject was observed to be perspiring profusely and in five minutes more reported dizziness sufficiently severe to warrant the termination of the experiment. When the records were examined quantitatively a decrease of 16 per cent in the extent of

the knee-jerk was found after the first treatment while after the second it was depressed 65 per cent.

It was found that the length of the spark gap varied with each subject. In some cases a very small spark gap was sufficient to produce 750 milliamperes, while in others a wide gap was required. Subject 4 received the full capacity of the machine which registered 1000 milliamperes.

In all cases except subject 9 the only effect observed or reported was profuse sweating. The reduction in the knee-jerk following auto-condensation treatment is explained by the fact that the heat generated in the tissues causes a decrease in tone which in turn is a relaxation of the muscle. This explanation is in accordance with the idea that the knee-jerk is an index to muscle tonus. It also falls in line with the work of Emery, who showed that an increase in temperature caused a decrease in tonus while a decrease caused increased tone. The work of Jacobson and Carlson (1925) supports the explanation of the results in this experiment. They found that

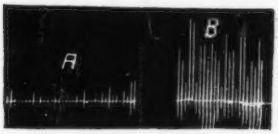

Fig. 1

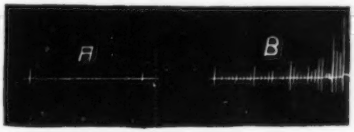

Fig. 2

Fig. 1. This is the record of subject 7. *B*, normal; *A*, after thirty minutes' treatment with auto-condensation current. The knee-jerk is reduced 91 per cent.

Fig. 2. This is the record of subject 2. *B*, normal; *A*, after thirty minutes' treatment with auto-condensation current. In this case the knee-jerk is reduced 96 per cent.

anything tending to cause relaxation was accompanied by a decrease in the knee-jerk.

An opportunity was not afforded at this time to study the duration of the effect of auto-condensation treatment. The writers plan to carry out such an investigation in the near future.

THE EFFECT OF HIGH FREQUENCY ELECTRIC CURRENT. In this experiment sixteen subjects were used. The method of procedure was to make a normal record of each subject as described above. As soon as this was done the point of impingement of the stimulus was noted and the subject removed from the chair. High frequency current was immediately applied to the muscles of the thigh by means of a glass electrode until the skin began to get red. At the completion of the treatment the subject was adjusted in the apparatus and a second record made. Table 2 shows the results of this experiment.

Figure 3 shows the effect of high frequency electric current on the knee-jerk.

TABLE 2

Comparison of the height of the knee-jerks of sixteen subjects before and after the muscles of the thigh were subject to high frequency electric current

SUBJECT	TOTAL HEIGHT OF KICKS	NUMBER OF STIMULI	AVERAGE HEIGHT OF KICKS		PER CENT OF DIFFERENCE
			Before	After	
	mm.		*mm.*	*mm.*	
1	885	42	21.07		
	4,861	144		33.75	60
2	785	29	26.89		
	4,509	80		90.11	235
3	80	64	1.25		
	298	104		2.86	129
4	1,567	37	42.35		
	3,757	70		53.67	27
5	1,087	34	31.97		
	3,100	54		57.40	79
6	28	30	0.90		
	121	70		1.72	91
7	395	41	9.63		
	716	34		21.05	119
8	260	28	9.29		
	761	60		12.66	36
9	2,168	59	36.91		
	11,051	117		94.44	156
10	1,036	42	24.66		
	5,855	164		35.70	45
11	1,484	49	30.29		
	7,498	142		52.80	74
12	505	32	15.62		
	2,685	59		45.50	191
13	4,649	135	34.43		
	4,539	73		62.17	81
14	2,018	39	51.74		
	6,145	83		74.03	43
15	1,070	32	33.43		
	3,399	79		43.02	28
16	565	59	9.58		
	1,153	97		11.88	24

It is seen from table 2 that without exception the knee-jerks of the group studied are increased after the thigh muscles have been subject to high frequency electric current. The extent of the increase is seen to depend on the individual subject varying from 24 per cent to 235 per cent.

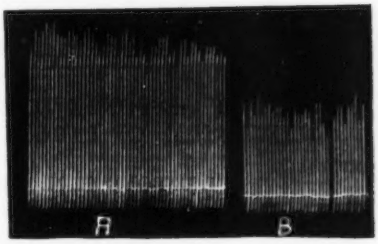

Fig. 3. This is the record of subject 5. B, normal; A, after 30 minutes' treatment with high frequency current. In this case the knee-jerk was increased 79 per cent by high frequency electric current.

It was noted that in order to produce marked effects it was necessary to cause a slight irritation of the skin. This observation leads the writers to believe that the increased tonus, after the application of high frequency current, as exhibited by the knee-jerk, is due to local irritation. There were no available means whereby the amount of high frequency current required could be measured.

No effects were noticed from the treatment except irritation and only in one case did this persist for more than a few hours.

CONCLUSIONS

D'Arsonval auto-condensation electric current, when applied in sufficient quantities to cause perspiration, reduces muscle tonus as exhibited by the knee-jerk.

The extent of the reduction depends upon the amount of current used.

High frequency electric current causes an increase in muscle tonus as exhibited by the knee-jerk when applied sufficient to cause irritation.

The writers wish to thank Mr. A. W. Mathis, representing H. G. Fisher & Co., for lending the electric equipment previously described.

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STUDY OF RESPONSE TO CONTINUOUS INTRAVENOUS INJECTION OF LARGE AMOUNTS OF GLUCOSE

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Preliminary to study involving probable derangements of the carbohydrate handling mechanism, it was deemed necessary to study more completely the response of normal animals to carbohydrate administration. This was done to determine the degree of variation in the same animal from time to time, and also in different animals under similar physiological conditions.

In the presence of gross lesions, such as depancreatization, extensive liver injury or phlorhidzin poisoning, the usual glucose tolerance test, based upon estimations of blood and urinary sugar content following carbohydrate administration, is perhaps adequate as a qualitative measure of the impaired function. However, when disturbances of lesser degree are to be measured, it is desirable that the study be as complete as conditions of the experiment permit.

The continuous intravenous method of administration was used in this work because of the desire to introduce at a constant rate larger quantities of glucose than absorption through the normal channel, the gastro-intestinal tract, would permit. It is well recognized that the rate of gastro-intestinal absorption is a variable factor (Beeler, Bryan, Cathcart and Fitz, 1922). The maximum rate of absorption has been estimated at about 1.8 grams per kilogram body weight per hour (Woodyatt, Sansum and Wilder, 1915).

The literature dealing with the intravenous method has been well reviewed by Kleiner (1916), Nonnenbruch and Szyszka (1920), Wilder and Sansum (1917), and Rigler and Ulrich (1923). A survey of this reveals that many different methods of intravenous administration have been employed. Some administer the whole amount in a single massive dose; others use frequent interrupted small quantities; still others have made use of special devices to maintain a constant intake over a long period of time. Of these, the electrically driven pump described by

[1] Reported in abstract before the Society for Experimental Biology and Medicine; Proceedings, xxii, p. 509.

Woodyatt (1915) is the most accurate; by its use one is able to inject continuously at almost any desired rate. The advantages of maintaining a uniform rate of flow is obvious when one compares the values for the minimum amounts of glucose required to produce glycosuria as obtained by the continuous and interrupted methods. Using this procedure, Woodyatt and his co-workers (1915), (1917), (1924) have reported studies on the response to glucose administration in man and lower animals, using in the latter cases wide ranges of quantities and concentrations. Their attention was chiefly directed, however, to a study of the urinary sugar and water balance. Little attention has been paid to blood sugar variations in cases where the intake was sufficient to produce glycosuria. Allen and Wishart (1920) have studied normal and depancreatized dogs, using the interrupted method for the most part. Albritton (1924) has recently reported blood sugar changes resulting from the administration of glucose in amounts less than that required to produce glycosuria.

Kleiner (1916) and MacLeod and Fulk (1917) have studied the response to large amounts of glucose intravenously, but their results have no doubt been modified by operative procedure and use of ether and morphine, each of which is known to affect the sugar-handling mechanism.

In this study glucose solutions have been administered continuously for considerable periods of time by means of a Woodyatt pump, and no anesthetic nor operative procedure employed other than venepuncture and catheterization. More complete concomitant studies have been made than by previous observers, including numerous observations on blood sugar, hemoglobin, volume and sugar content of urine, plasma pH and CO_2 content, body temperature, respiratory quotient and heat production before, during and following the glucose administration. In addition, the physiological conditions of the animals have been controlled by administration of a constant diet during periods preceding observation, and by comparing the response of the same animal to repeated injections.

Methods. Dogs were placed on a standard diet essentially the same as that employed by Lusk (1912) in his experimental work. No food was given for 18 to 20 hours preceding the injection. Animals were accustomed to cage life and laboratory procedure before attempting observations. Preliminary to injection, estimations were made of all factors followed during the experiment, to serve as controls. Blood for sugar and hemoglobin determinations was obtained from the marginal ear vein, the ear having been shaved, and the vein opened by a longitudinal slit with a razor blade. For pH and CO_2 content samples were obtained in the usual manner from the jugular or saphenous vein. The animal was then catheterized, and the needle inserted into the saphenous vein, being held in place by means of strips of adhesive tape encircling the leg. In-

jection was made according to the method of Woodyatt, the pump being regulated to deliver the solution at such a rate that the animal would receive approximately 4 grams glucose per kilo body weight per hour. Frequent readings of the burette were made in order to insure proper administration rate. A 30 per cent solution of C. P. glucose (Merck) was used, the concentration checked by chemical analysis. In most cases Locke's solution was used as solvent, although Ringer's solution and distilled water were used in some cases, to determine whether the pH of the solvent would alter the response. When Locke's solution was used, the sodium bicarbonate was added after sterilization, and gave the solution a pH slightly less than 7.4.

Urine sugar was determined according to the method of Folin and Berglund (1922), suitable dilutions being made in each case. Samples were considered sugar-free if they failed to react with Benedict's qualitative reagent. Quantitative studies were not made of smaller concentrations.

Hemoglobin was determined according to the method of Newcomer (1919). The preliminary value was used as a standard for the later readings, being designated as 100 per cent.

Blood sugar was determined according to a method based on that of Folin and Wu (1920). One-fifth cubic centimeter of blood was added to 4 cc. distilled water in a centrifuge tube; then the proteins were precipitated by the addition of $\frac{2}{3}$ normal sulfuric acid and 10 per cent sodium tungstate, 0.4 cc. of each. After mixing and centrifuging, the filtrate was used as described by Folin and Wu, except that the final dilution was to 10 cc. instead of 25 cc. Where there was marked hyperglycemia, only 1 cc. of filtrate was used, an equal quantity of water being added to the sugar tube to make up the proper volume. Terminal dilution in these cases was sometimes made to 25 cc.

The plasma CO_2 content was determined according to the method of Van Slyke and Cullen (1917). For pH determination, the method of Myers, Schmitz and Booher (1923) was employed.

Air was collected in the Tissot spirometer. The collecting mask was similar to that employed by Kitchen (1924). In most cases the mixed sample represented the total expired gas during a period of 10 to 15 minutes. It was analyzed according to the Haldane method.

The period of observation after injection averaged about eight hours, in some cases much longer. After its completion the animal was returned to its cage and given its diet. This was completely eaten unless otherwise noted in the protocols. In some animals injections were made on successive days, in others on alternate days. In some cases considerable time elapsed between successive injections.

Results. The period of injection was associated with a characteristic

readjustment of the physiological equilibrium of the animal. After injection there was a rapid return to the preliminary condition, insofar as could be determined by the methods of study employed.

Variations in blood sugar values during the period of observation were quite consistent in all cases. In general, they may be summarized as follows:

Preliminary values (18 hours post-absorptive) approximated 0.1 per

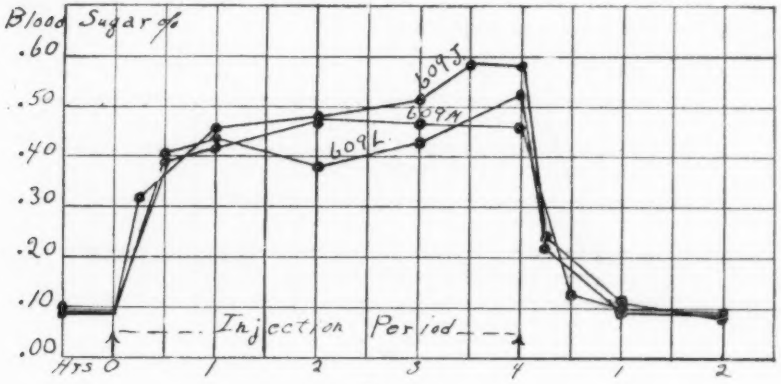

Fig. 1

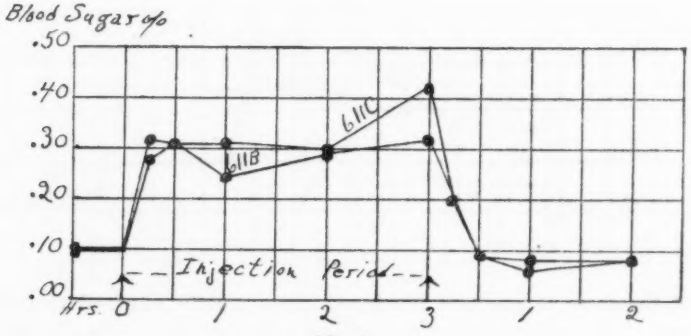

Fig. 2

cent. The average for all cases was 0.099 per cent; the extremes were 0.076 to 0.119. When injection was instituted, there was a rapid rise in concentration; this was quite uniform for the first fifteen minutes, at the close of which period it averaged 0.29 per cent. During the first hour a first maximum was observed in over 30 per cent of the cases; it is possible that more frequent sampling during that period would have shown its incidence to be greater. Following the initial rapid rise in

concentration, the blood sugar curve tended to assume the form of a plateau, in some cases tending to gradually rise, in others to gradually decline. With the more prolonged injections, a terminal rise was often seen during the last hour. The average blood sugars found in the series presented for the 1st, 2nd, 3rd and 4th hour samples were 0.371, 0.376, 0.390 and 0.430, respectively. The response of the same animal to repeated injections was more constant, the maximum variations being 0.22 per cent, 0.15 per cent, 0.18 per cent and 0.12 per cent, for the 1st, 2nd, 3rd and 4th hours respectively.

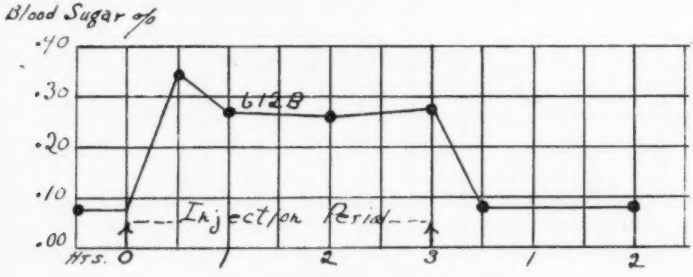

Fig. 3

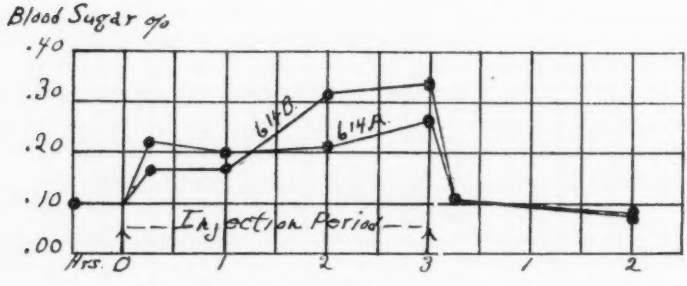

Fig. 4

At the close of injection the blood sugar rapidly returned to normal values. The constancy and rate of this fall is similar to the rate of rise during the first few minutes of injection. In most cases the preliminary value had been reached or passed within the first thirty minutes. Only two cases failed to show some degree of hypoglycemia at some period during recovery. While this was variable in degree, it was comparable to that frequently observed following alimentary glucose administration. The blood sugar variations are shown graphically in the accompanying charts, repeated experiments on the same animal being superimposed.

During the period of injection, glycosuria was a constant finding in

all cases. This is to be expected, because in all cases pronounced hyperglycemia was present. Sugar disappeared from the urine promptly upon termination of the injection, usually within the course of the first hour. The percentage of retention of the injected glucose varied considerably in different animals, averaging 79.1 per cent, the limits of variation being from 61.4 to 87.8 per cent. In the same animal, however, the degree of constancy is much more marked. Five animals out of seven

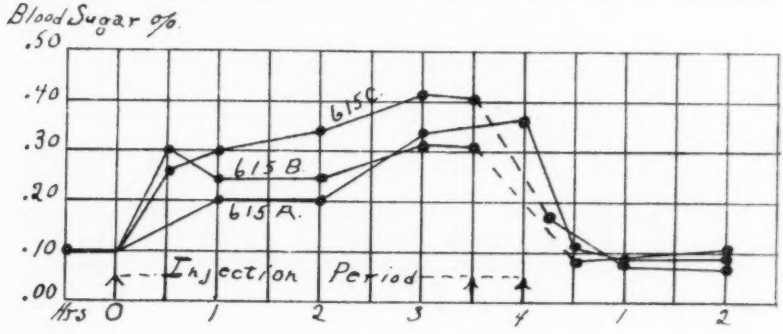

Fig. 5

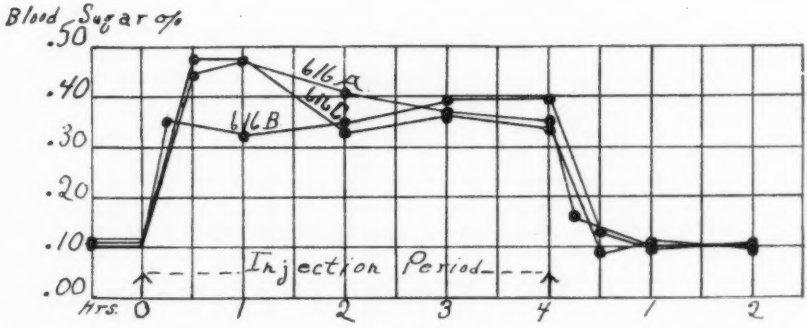

Fig. 6

showed upon repeated injections individual variations in percentage of glucose retained of from 1.1 to 5.9 per cent. We noted no evidence of cumulative effect of repeated injections, insofar as degree of sugar retention is concerned. This was equally true in an animal receiving injections at daily intervals and one receiving the second injection after a lapse of a month or more.

The glucose was administered in Locke's solution in most instances. A comparison of the response was made when distilled water, Locke's

or Ringer's solution was used, and no significant difference between them could be discerned.

The term sugar retention is used to represent that part of the sugar injected which does not appear in the urine during injection nor the

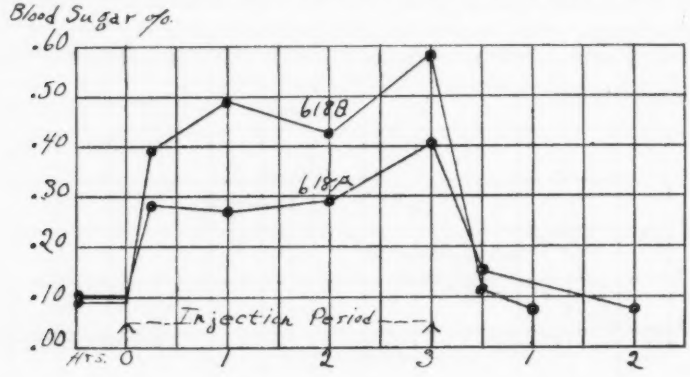

Fig. 7

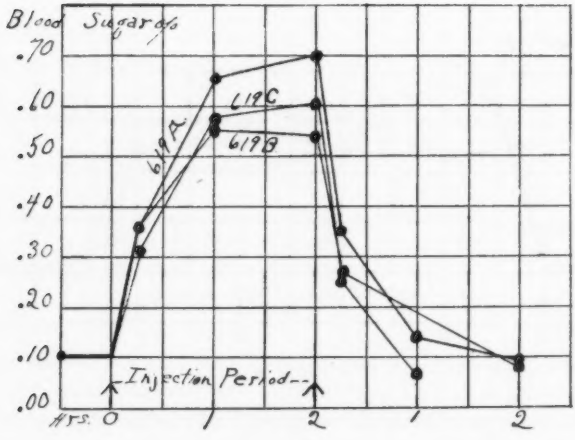

Fig. 8

recovery period. This includes the amount oxidized, retained in the blood and tissues, and any which may be excreted into the gastro-intestinal tract to be subsequently reabsorbed. The experiments of Kleiner (1911) would indicate that the latter quantity is small.

The percentage retained at the end of hourly periods during injection

TABLE 1

Percentage of injected glucose retained—based upon the percentage of total amount injected and eliminated up to the designated time

EXPERIMENT NUMBER	END OF FIRST HOUR	END OF SECOND HOUR	END OF THIRD HOUR	END OF FOURTH HOUR	TOTAL
609J	94.0	89.9	86.6	86.1	83.7
609L	90.0	86.8	86.4	84.5	84.1
609M	87.0	92.3	81.4	78.9	78.4
Deviation.....	7.0	5.5	5.2	7.2	5.7
611B	93.3	92.7	88.0		87.3
611C	86.3	87.2	83.0		82.0
Deviation.....	7.0	5.5	5.0		5.3
612B	93.9	89.5	88.7		87.8
614A	85.7	85.8	83.5		82.9
614B	94.1	87.2	84.0		84.0
Deviation.....	8.4	1.4	0.5		1.1
615A	93.2	91.4	89.2	84.5	83.8
615B	89.3	86.9	84.2		84.6
615C	87.7	84.8	78.8		77.9
Deviation.....	5.5	6.6	10.4		5.9
616A	83.3	76.9	77.2	77.9	77.6
616B	88.8	83.9	79.0	75.6	75.1
616C	83.4	81.8	80.2	76.2	76.5
Deviation.....	5.5	7.0	3.0	2.3	2.5
618A	91.6	85.2	82.7		82.4
618B	74.4	78.6	69.4		68.3
Deviation.....	17.2	6.6	13.3		14.1
619A	77.4	66.3			61.4
619B	79.7	75.3			73.0
619C	79.5	74.7			73.0
Deviation.....	2.3	9.0			11.6
Average.....	86.9	84.1	82.6	80.5	79.1

tends to gradually decrease during the course of the injection. In a few cases, however, greater retention was observed the second hour than the first. In most cases the degree of sugar loss in the urine was somewhat

proportional to the height of the hyperglycemic plateau. This relationship is more consistent when successive injections of the same animal are compared rather than when different animals are compared. Thus dog 609 consistently showed a higher plateau than dog 616, yet retained a greater per cent of the injected sugar. (See table 1.)

The sugar percentage in the urine showed no marked degree of constancy in the various experiments. During the course of an injection, however, the concentration of the sugar gradually increased in proportion to the increase in sugar output. Variations of concentration in different injections have no apparent relationship to the percentage of sugar retained.

TABLE 2

Blood plasma pH, CO_2, and hemoglobin relationship—determined before and at end of the injection

EXPERIMENT NUMBER	pH BEFORE	pH AT END	DIFFERENCE	CO_2 BEFORE*	CO_2 AT END	DIFFERENCE	HEMOGLOBIN AT END†
609J	7.38	7.32	−0.06	48	33	−15	108
609L	7.37	7.29	−0.08	54	39	−15	85
609M	7.37	7.33	−0.04	51	46	−5	95
612B	7.32	7.30	−0.02	51	38	−13	105
614A	7.38	7.34	−0.04	47	37	−10	100
614B	7.36	7.33	−0.03	45	38	−7	104
616B	7.32	7.29	−0.03	46	41	−5	94
616C	7.38	7.37	−0.01	43	41	−2	89
619A	7.30	7.30	0.00	46	34	−12	86
619B	7.39	7.35	−0.04	44	40	−4	82
619C	7.36	7.31	−0.05	45	38	−7	85
Average.....	7.357	7.32	−0.036	47.3	38.6	−8.7	

* CO_2 recorded in volume per cent.

† Initial value of hemoglobin = 100 per cent.

The preliminary plasma pH values were quite constant, usually about 7.37, and averaging 7.36 for the series. These values were found to be slightly lowered at the end of injection in most cases. In thirteen experiments on five animals the drop of pH ranged from 0.00 to 0.08, averaging 0.036.

The CO_2 content was determined in a portion of the plasma from each of the above samples. Preliminary values were always within the normal range, usually about 45 to 50 volumes per cent, averaging 47.3 in all samples tested. At the end of injection there was found a drop in the CO_2 content in all cases, varying in extent from 2.0 to 15.0 volumes per cent. (See table 2.)

Respiratory analysis revealed that the period of injection was accom-

panied by increased heat production in every case. The average increase in the samples obtained during injection as compared with the preliminary values was 48 per cent. There appeared to be but slight fluctuation of heat production during injection. When one injection is compared with another, however, there is considerable variation between the levels of heat production attained.

There is a rapid fall of heat production when injection is terminated, the average value an hour after injection being about 13 per cent above the preliminary figure. In most cases normal level had been resumed

TABLE 3

Calories per square meter of body surface per hour

Based upon Rubner's constant in Meeh's formula—11.2 $\sqrt[3]{\text{weight in grams}^2}$

EXPERIMENT	PRELIMINARY	INJECTION PERIOD			RECOVERY PERIOD		
		AVERAGE	Per cent deviation over preliminary rate	Per cent deviation over average preliminary rate	0 to 1 hour	1 to 2 hours	2 to 4 hours
			per cent	*per cent*			
609J	52.8	65.0	23	50	45.1	42.8	41.8
609L	43.9	76.7	75	76		43.4	40.9
609M	44.7	61.9	39	43	48.7		40.5
611B	43.0	77.2	79	78		59.8	63.7
612B	46.7	64.8	39	50	47.8		57.2
614A	45.6	57.3	26	32	59.3	45.5	
616A	45.9	61.8	35	43	47.6		41.3
616B	41.8	62.5	50	44		55.0	40.2
616C	42.5	64.5	52	49		49.2	38.0
618A	37.6	67.6	80	56			
618B	43.7	65.9	51	52		44.0	
619A	38.4	52.7	37	22	52.1		
619B	38.9	56.7	46	31	47.2		
619C	41.8	60.5	45	40	55.4		
Average.....	43.3	63.9	48.3	47.6	50.4	48.5	45.5

within three hours after injection was terminated. The respiratory quotient returned to normal more gradually.

The preliminary quotients are quite consistent, averaging 0.81. The values obtained during injection were quite constant, averaging 0.978. There was but little fluctuation during the period of injection, the hourly averages being 0.973, 0.971 and 0.989 for the 1st, 2nd and 3rd–4th hour samples respectively. Only five values over 1.00 were noted. After injection was discontinued, there was a gradual return to the preliminary quotient level, hourly averages being 0.919, 0.883, 0.864, 0.848, for the 1st, 2nd, 3rd and 4th hours respectively, and 0.842 for values obtained

TABLE 4

Respiratory quotients and calories per dog

Figures in parentheses refer to the time in hours relative to start and end of injection

EXPERIMENT		PRELIMINARY	INJECTION PERIOD			RECOVERY				LENGTH OF INJECTION
										hours
609J	R. Q.	0.78	0.95(1)	0.94(3)	0.98(4)	0.94(1)	0.83(2)	0.72(4)		4
	Calories	33.37	45.23	40.42	47.49	28.48	27.03	26.39		
609L	R. Q.	0.77	0.85(2)	0.90(4)		0.85(2)	0.82(3)			4
	Calories	27.49	47.76	48.25		27.11	25.60			
609M	R. Q.	0.80	0.94(2)	1.00(4)		0.92(1)	0.90(3)	0.86(7)		4
	Calories	28.56	42.0	37.17		31.12	25.86	25.94		
611B	R. Q.	0.85	0.93(1)	0.95(2)	0.95(3)	0.74(2)	0.83(4)	0.80(7)		3
	Calories	23.82	46.91	43.12	38.22	33.50	35.30	28.64		
612B	R. Q.	0.79	0.96(1)			0.95(0)	0.78(1)	0.85(3)		3
	Calories	27.15	37.64			30.31	25.14	33.21		
614A	R. Q.	0.82	1.02(1)			0.98(1)	0.89(2)	0.83(6)		3
	Calories	24.49	30.76			31.84	24.43	24.54		
616A	R. Q.	0.82	0.95(2)	1.00(4)		0.93(1)	0.85(3)			4
	Calories	20.21	27.29	27.13		20.99	18.18			
616B	R. Q.	0.81	0.98(2)	0.92(4)		0.92(2)	0.90(4)	0.89(7)	0.81(11)	4
	Calories	18.39	27.70	27.36		24.22	17.69	21.24	21.37	
616C	R. Q.	0.80	0.98(2)	1.04(4)		1.01(2)	0.94(4)	0.83(9)	0.75(21)	4
	Calories	19.68	30.97	28.70		22.76	17.58	14.95	20.38	

618A	R. Q.	0.82	0.95(1)	1.00(3)	0.90(3)	0.80(6)		3
	Calories	24.00	42.88	43.51	31.08	25.84		
618B	R. Q.	0.85	0.98(2)	1.16(3)	0.94(2)	0.85(20)		3
	Calories	28.42	45.19	40.26	28.63	26.17		
619A	R. Q.	0.82	0.98(2)		0.87(0)	0.79(5)		2
	Calories	25.37	34.79		34.39	26.30		
619B	R. Q.	0.80	0.99(1)	1.12(2)	0.98(1)	0.87(7)		2
	Calories	25.66	38.67	36.12	31.12	24.80		
619C	R. Q.	0.81	1.01(1)	0.98(2)	0.92(1)	0.86(6)		2
	Calories	27.60	41.25	38.54	35.20	25.36		

during the 5th to 7th hour. It was noted that when the same animal received several injections at intervals of one or two days, the return of the respiratory quotient to the preliminary level was delayed in the successive experiments (see tables 3 and 4).

In the presence of the marked diuresis associated with injection, it was felt that attempts to estimate the non-protein respiratory quotient would be inaccurate. Such measurements were therefore not made.

The degree of water loss, calculated from the ratio of intake to output, was relatively small. Hemoglobin values at the end of the injection period, shown in table 2, indicate no consistent changes in blood volume.

It will be noted in the protocols that the average increment of temperature rise during injection was practically one degree centigrade.

DISCUSSION AND CONCLUSIONS. These experiments were designed primarily to test the adequacy of the sugar handling mechanism in normal animals. This required that the animal be subjected to a greater demand for sugar storage than that furnished by the maximum rate of physiological absorption. The strain should not be sufficient, however, to severely overtax the organism. An administration rate of 4 grams per kilo body weight per hour was chosen tentatively, and has seemed to serve the necessary qualifications.

Woodyatt, Wilder and Sansum (1915) have established that by the intravenous route, man and animals can tolerate glucose administration at the rate of 0.85 gram per kilo per hour for hours. This seems to be the upper limit of utilization without glycosuria in man, although animals sometimes tolerate quantities somewhat greater. They found that an injection rate of 1 gram per kilo per hour caused glycosuria in all cases.

The maximum rate of absorption is frequently given as about 1.8 grams per kilogram per hour (Woodyatt, Wilder and Sansum, 1915). As has been stated, the degree of absorption is not constant at all times nor in all conditions, and therefore cannot be relied upon.

To determine the degree of constancy of response in a given animal to daily injections according to the method employed in these experiments, it is necessary to terminate the injection soon enough to permit its resumption with normal preliminary values on the following day. We have found that an injection period of three or four hours accomplishes this, and also provides pronounced increases in storage forms. The latter factor is desirable where tissue analysis is to be performed.

These experiments have apparently had no deleterious effects on the animals employed. This is evidenced by the fact that three successive daily injections have not essentially altered the response, as judged by the methods employed in this work. Animals showed no noteworthy toxic symptoms during or after the injection. Following injection there has been a rapid return to preliminary values of blood sugar concentration,

heat production, respiratory quotient and associated factors. No marked changes were noted in blood volume or water balance. In most cases animals showed moderate gain in weight and were apparently in good health two months after the experiments were complete. We do not feel, however, that this is any evidence that a marked polyuria resulting from glycosuria or prolonged hyperglycemia may not produce pathological changes if persistent for long periods of time.

For any one animal subjected to repeated injections, the response under similar conditions of diet, activity and external environment is quite constant throughout. Considerable variation seems to exist, however, in the responses of different animals under similar physiological conditions. Moreover, these variations do not always involve similarly all the various factors involved. This is of interest in view of Woodyatt's interpretation of glucose tolerance as measured by the degree of glycosuria associated with intravenous injection.

Allen and Wishart (1920) have criticized the acceptance of degree of glycosuria as an adequate measure of glucose tolerance, because of varying degrees of renal permeability. They consider the intravenous method of determination of glucose tolerance to be fallacious unless both glycosuria and blood sugar concentrations are observed. Our results would indicate a greater variation in the percentage increase of blood sugar during injection than the degree of glycosuria obtained. While in any one animal the variations in blood sugar are roughly parallel to fluctuations in glycosuria, the variable plateaus of blood sugar values in different animals would lead to error and confusion if used as a sole index as to efficiency of response.

Respiratory gas measurements were made for two purposes; first, to ascertain if possible the effect per se of the injection of glucose on the respiratory quotient and heat production, as to possible indications of fat formation, and as to degree of effect of carbohydrate plethora; secondly to ascertain the approximate quantity of carbohydrate oxidized during the experiment, in order to observe quantitatively the fate of the glucose administered.

Analysis of the respiratory data reveals a quite constant preliminary respiratory quotient. The preliminary heat production values, although within the range reported by Kitchen (1924) were higher than those which others have obtained by direct calorimetry. Subsequent determinations and longer training periods do not seem to appreciably lower these values. We believe that in most cases the increment is due to increased muscle tonus associated with the conditions of the experiment. For this reason we prefer to speak of our initial heat values as preliminary, rather than basal. The increase of respiratory quotient obtained (when proper consideration has been given to overventilation and alteration

of the plasma CO_2 content) would indicate increased utilization of carbohydrate rather than fat formation.

An increase in degree of heat production is associated with the period of injection. Since the preliminary values are not necessarily basal, it is not justifiable to ascribe all of the increment to the stimulating action of carbohydrate plethora. Moreover, tension and tonus may be increased during the experiment. The small but consistent rise in body temperature during injection may increase the heat production, as has been pointed out by DuBois (1924). The magnitude of such effect, however, would probably be less in this association than when due to the products of toxemia. It is possible that the usual increment of heat production associated with carbohydrate plethora may be exceeded when the rate of assimilation is more than doubled, as is the case in these experiments. That the increase in heat production is not the result of some toxic action produced by the injection is rendered improbable, because of the rather prompt return to normal upon cessation of injection.

It is difficult to differentiate between the effects of the glucose injection per se and those produced by the binding of tissue fluids due to the retention of glucose in the tissues. The rise in temperature is analogous to the sugar- or salt-fever, noted when crystalloids are injected into the blood stream faster than they can be cared for by compensatory measures. Such a condition may be produced, and yet neither changes of weight nor diuresis indicate loss of body fluids. This type of masked anhydremia can produce diminution of the alkali reserve, which Clausen (1925) has shown to be associated in many cases with increased lactic acid concentration in the blood. The condition disappears promptly when normal oxidation again becomes possible. In some cases this might be ascribed to circulatory embarrassment; yet in these experiments there was at no time any indication of such state.

The cause for the slight variations found in the same animal during repeated injections, and the still greater variations found in different animals, is not apparent. Early in the course of this work, sufficient attention was not paid to the preliminary condition of the animal as to diet and previous activity. While the work at that time involved much less extensive data collection, the values obtained showed but slight consistency in degree of response of the same or of different animals. We believe that if much more care be exercised in the selection of animals for metabolic study, a more constant and consistent response will be obtained. This would involve a knowledge of the age, breed, occurrence of previous infections, previous diet and environment. Lusk (1915) has noted the marked effect of cage life on basal metabolism. There is no reason to doubt that other factors are not similarly affected by such conditions.

In the light of the present investigation and those of others, we believe that when conditions such as previous diet, physical soundness and environment are properly controlled, the intravenous injection of glucose in amounts larger than those obtainable by alimentary absorption will furnish a delicate means of testing the carbohydrate capacity of an animal. However, more care must be exercised in selection of animals for objects of study, for considerable variation exists between dogs selected at random. The normal response of an animal should be established before attempting to estimate small changes in glucose metabolism. Moreover, as many factors as possible should be correlated before attempting to interpret the carbohydrate-handling efficiency of the organism.

SUMMARY

1. Coincident with the intravenous injection into dogs of glucose solution continuously at a rate of 4 grams per kilogram body weight per hour, studies have been made of fluctuations of glycosuria, blood sugar, plasma pH and CO_2 content, hemoglobin, respiratory quotient and heat production. The injection period was from two to four hours.

2. The response of a given animal to repeated injections is quite uniform, if performed under similar physiological conditions.

3. The response is qualitatively similar in different dogs, but quantitative relationships show considerable variation.

4. Blood sugar rises quite rapidly during the first few minutes of injection, then tends to assume a plateau. The height attained is not an index of the degree of associated glycosuria. After injection, blood sugar rapidly resumes normal concentration. About 20 per cent of the injected glucose appears in the urine.

5. Heat production is increased about 50 per cent during the injection; this rapidly subsides to preliminary value after injection is terminated. The respiratory quotient indicates predominant glucose combustion, but offers no positive evidence of fat formation.

6. The injection is associated with a slight decline in plasma pH and CO_2 content; this apparently bears no relation to the pH of the injected fluid.

7. A slight rise in body temperature was associated with the injection period.

8. It is proposed to use the procedure described in a study of slight alterations of glucose metabolism.

We wish to express our appreciation for the kind coöperation of Dr. J. T. McClintock in this work.

PROTOCOLS. *Expt. 609J.* Young bull terrier, male, weight 13.2 kgm., in good nutritional condition. Preliminary period of cage life and standard diet. On March 17, 1925, injected for 4 hours with 679 cc. of a 31.25 per cent glucose solution in buffered Ringer's solution by means of a Woodyatt pump. Tendency to shiver and pant during course of gas collections. At end of injection period animal had passed 760 cc. of urine and had a rise of 0.6°C. of rectal temperature.

Expt. 609L. Same animal as above (609J). Preliminary conditions similar, weight 13.2 kgm. On April 7, 1925, injected 4 hours with 734 cc. of a 29.4 per cent glucose solution dissolved in double distilled water. At end of injection had passed 690 cc. urine and rectal temperature had risen 1.1°C. Was slightly restless at times; vomited 45 minutes after end of injection.

Expt. 609M. Two days after experiment 609L injected same animal with 727 cc. of 28.75 per cent glucose dissolved in Ringer's solution. During injection passed 1015 cc. urine, and rectal temperature rose 0.6°C.

Expt. 611B. Female Airedale dog, weight 11.0 kgm., in good nutritional condition. On day previous to injection had received 16.5 units insulin intravenously. On February 17, 1925, injected 448 cc. of 32.5 per cent glucose solution in Locke's solution, during period of 3 hours. Animal was restless throughout injection; passed 464 cc. of urine, and rectal temperature rose 0.7°C.

Expt. 611C. Same animal as in experiment 611B. Injected 3 hours, receiving 407 cc. of 32.5 per cent glucose in Ringer's solution. Because of tension, air samples discarded. Passed 425 cc. urine, and rectal temperature rose 0.4°C.

Expt. 612B. Adult female dog, weight 11.36 kgm. Eskimo breed, in good nutritional condition. On March 5, 1925 injected for 3 hours 13 minutes, receiving 425 cc. of 30.5 per cent glucose in Locke's solution. Passed 480 cc. of urine, and rectal temperature rose 1.8°C.

Expt. 614A. Young female collie, weight 10.5 kgm. On June 8, 1925, during injection of 3 hours with 33.4 per cent glucose in Locke's solution, received 390 cc. Passed 280 cc. urine, and rectal temperature rose 0.9°C.

Expt. 614B. Two days after experiment 614A, same animal during injection of 3 hours with 33.3 per cent glucose in Locke's solution, received 387 cc. Passed 180 cc. urine, and rectal temperature rose 1°C. Because of shivering, respiratory determinations were discarded.

Expt. 615A. Adult female Spitz, weight 7.4 kgm. In good nutritional condition. Because of nervousness due to wearing of mask, no calculations of respiratory data were made. On March 25, 1925, was injected for 4 hours with 29.6 per cent glucose in Locke's solution, receiving 406 cc. During injection and first three hours of recovery period, passed 487 cc. urine, and rectal temperature rose 0.2°C.

Expt. 615B. Next day; injected 366 cc. of 29.6 per cent glucose in Locke's in 3 hours 33 minutes. During injection and first two hours of recovery period, passed 350 cc. urine, and rectal temperature rose 0.8°C.

Expt. 615C. Next day after experiment 615B, injected 378 cc. of 29.6 per cent glucose in Locke's solution during 3 hours 33 minutes. Passed 490 cc. urine, and rectal temperature rose 0.9°C.

Expt. 616A. Adult terrier, female, weight 7.8 kgm., rather thin. On April 10, 1925, received 419 cc. of 30.5 per cent glucose in Ringer's solution during 4-hour period, during which rectal temperature rose 0.5°C. Two hours after injection closed, total urine output had been 625 cc.

Expt. 616B. On next day, same animal received during 4 hours 418 cc. of 30.5 per cent glucose in distilled water. Rectal temperature rose 1.2° during injection. Two hours after close of injection, total urine output had been 435 cc.

Expt. 616C. Two days later, same animal received during 4 hour injection, 419 cc. of 30.5 per cent glucose in Locke's solution. Rectal temperature rose 1.2°C. Two hours after close of injection, total urine output had been 480 cc.

Expt. 618A. Young shepherd mongrel, nullipara, weight 13.6 kgm. Had mange. On June 3, 1925, during 3 hour injection, received 535 cc. of 31 per cent glucose in Locke's solution. Rectal temperature rose 2.1°C. 2 hours after close of injection, total urine output had been 560 cc.

Expt. 618B. Next day, same animal received 541 cc. of 31 per cent glucose in Locke's solution during 3 hours, and rectal temperature rose 1.6°C. One hour later, total urine output had been 780 cc.

Expt. 619A. Female collie, multipara, weight 14.3 kgm. in heat. On June 9, 1925, injected 347 cc. of 33 per cent glucose in Locke's solution in 2 hours; rectal temperature rose 0.8°C. 2 hours after injection, total urine output had been 666 cc.

Expt. 619B. Two days later, received 339 cc. of 33.4 per cent glucose in Locke's solution in 2 hours. Rectal temperature rose 0.9°C. Two hours later, total urine output was 461 cc.

Expt. 619C. Two days after experiment 619B, received in two hours 341 cc. of 33.3 per cent glucose in Locke's solution. Rectal temperature rose 1°C. Two hours later, total urine output had been 470 cc.

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THE DISTRIBUTION OF VITAMIN B IN THE MAIZE KERNEL[1]

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The relatively large part played by the cereal grains in total food consumption gives them a place of great importance in animal and human nutrition. Numerous studies on the occurrence of vitamins in various foods have shown that the cereals contain comparatively abundant supplies of vitamin B. Since rice polishings and wheat embryo are exceedingly rich in this accessory factor, it has been generally assumed that vitamin B resides chiefly in the embryo and perhaps to a less extent in the peripheral layers of these seeds. Contrary to this generally held opinion, Osborne and Mendel (1919) showed that vitamin B is not confined to any one part of the *wheat* kernel, but is distributed throughout this entire grain. Their observations were later confirmed by Bell and Mendel (1922).

Although the *maize* kernel as a whole contains abundant antineuritic vitamin, the highly-milled corn meal from which a large part of the bran and germ has been removed in modern milling processes, is deficient in this factor (Voegtlin, Sullivan and Myers, 1916; Weill and Mouriquand, 1917; Voetlin, Lake and Myers, 1918; Voegtlin and Myers, 1918a, 1918b; Green, 1918a, 1918b; Jansen, 1923).

Voegtlin, Sullivan and Myers (1916), basing their conclusions on an investigation of milling products of corn and wheat, stated that the antineuritic vitamin is "located in the intact kernel in the outer layers (aleurone layer) and probably also in the germ." According to Voegtlin, Lake and Myers (1918), this vitamin "seems to reside in the peripheral layers and the germ of the seeds, while the endosperm is relatively poor in this substance." Jansen (1923), studying the antineuritic vitamin in milling products of maize, gave his opinion that "for maize, the same holds true as for rice and other cereals, that the vitamin is found chiefly in the outer layers, i.e., in the part worn away by polishing."

Voegtlin and Myers (1919), experimenting with grains from which the germs

[1] The data in this paper are taken from a dissertation presented by Hilda M. Croll in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Yale University, 1925.

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had been removed by hand-cutting, concluded that "the germ or embryo of the wheat and corn kernel contains *all* of the antineuritic vitamine of these cereals," and (1920) "the aleurone cells are not the seat of the antineuritic vitamine, and this substance resides in the portion containing the germ, and probably within the germ."

Chick and Hume (1917a; 1917b) showed that the embryo of the maize grain, when separated from the soaked kernel by hand-dissection, possesses marked antineuritic properties.

Funk and Dubin (1920), using the yeast-growth method for quantitative study of water-soluble vitamin, found that "corn and wheat, separated from the germ by hand by Voegtlin and Myers' method, still exhibited considerable activity, although about ten times less than that shown by the germ-containing portion."

Most of these studies were concerned with the antineuritic vitamin. In view of the increasing doubt of the identity of this factor with the growth-promoting vitamin B, and because of the existing difference of

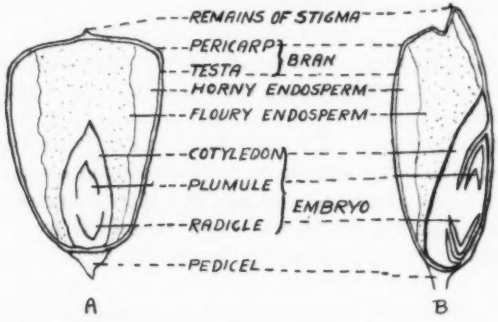

Fig. 1. Drawings (diagrammatic) showing the main structural divisions of the maize kernel. *A*, longitudinal section parallel to the flat surfaces of the grain. *B*, longitudinal section perpendicular to the flat surfaces of the grain. These drawings were adapted from illustrations in Bailey (1914), Burtt-Davy (1914), Weatherwax (1923) and Winton (1906).

opinion as to the exact location of these substances in the cereal grains, further investigation of this subject seemed desirable. Accordingly an inquiry into the distribution of vitamin B in several of the cereal grains was undertaken, this paper presenting an account of the results obtained on the maize kernel.

STRUCTURE OF THE MAIZE KERNEL. The maize kernel (fig. 1) is covered with a fibrous outer skin or hull (bran), which is the combined pericarp (wall of the ovary) and testa (seed-coat); the pericarp is histologically modified as it extends downward to the base of the seed, forming the spongy pedicel ("tip-cap") of the fruit, at the point of attachment of the grain to the ear. Beneath the hull is a single layer of uniform, cubical, aleurone cells, which form a sort of false epidermis for the endosperm. The hull and aleurone layer enclose the main mass of the kernel, the

endosperm and the embryo or germ. The embryo comprises the essential organs of growth,—the radicle or primary root, the cotyledon or seed-leaf, and the plumule or young shoot, which develops into stem and leaves. The embryo lies at one side of the endosperm, its position being marked by a slight oval depression in the grain; as the grain stands on the ear, the germ lies on the upper side of the kernel, facing the tip of the ear. The endosperm contains a store of food material, chiefly starch grains embedded in a net-work of protein and some cellulose, although in the sweet and waxy varieties of corn, sugar and dextrins are present in considerable amounts also. In the peripheral regions the endosperm is hard and flinty (horny endosperm), the spaces between the starch grains being filled with a dense matrix of protein and colloidal carbohydrate; in the interior of the endosperm and always in contact with the cotyledon of the germ, there is a region of softer, more opaque, floury endosperm, richer in starch, and containing less protein in the interstices of the starch grains. As the germinating embryo develops, it absorbs this stored food from the endosperm through a special organ, the scutellum, lying against the soft floury endosperm.

EXPERIMENTAL TECHNIC. The tests for vitamin B were conducted on healthy, young albino rats during the period of rapid growth from 40 to 50 grams to 140 to 150 grams body weight. The animals were kept individually in cylindrical wire-mesh cages, described by Ferry (1920); they were weighed twice each week, the weekly food intake also being determined.

Mixtures of purified food substances were used to furnish a diet adequate except for the lack of vitamin B; so that when the latter was supplied in sufficient amount, satisfactory growth of rats could be attained with the food. The diets were based on "standard" mixtures of the following composition:

"Standard" Food 1 and 2	*per cent*	*"Standard" Food II*	*per cent*
Casein 1 or 2*	21	Casein (Harris)*	18
Cornstarch	48	Cornstarch	51
Salt mixture†	4	Salt mixture†	4
Lard	22	Lard	22
Cod liver oil‡	5	Cod liver oil‡	5

* Since the lots of commercial casein, 1 and 2, contained only 12.75 per cent of nitrogen, diets including them were made up with 21 per cent of the product; only 18 per cent of the purer Harris casein, free from vitamin B, was included in the food.

† The salt mixture described by Osborne and Mendel (1917) was employed.

‡ The cod liver oil used in this study was furnished by the Harris Laboratories, Tuckahoe, New York.

Two procedures were adopted in testing for vitamin B. In certain cases the "standard" food mixture was supplied *ad libitum* to rats, vitamin

B being furnished in weighed quantities of ground whole corn, corn embryo, endosperm or bran, apart from the rest of the ration. By this procedure it was possible to compare the vitamin content of the different parts of the corn kernel. In control experiments dried brewery yeast was used as a source of vitamin B. In other experiments definite proportions of ground whole corn, endosperm or embryo were incorporated into the paste food, the corn product in each case being substituted for an equal quantity of starch in the "standard" mixture.

The lowered food intake, due probably to loss of appetite, resulting when a food poor in vitamin B was incorporated into the "standard" diets, introduced the vicious circle in which, as the appetite became poorer there was in turn less food and vitamin eaten, and therefore less stimulation to the appetite. For this reason, the separate feeding of the vitamin-bearing material is a preferable procedure, since by this method the rats receive quantitatively definite amounts of vitamin daily.

Typical curves of changes in body weight of some of the experimental animals are shown in the accompanying charts,[2] together with reproductions of Donaldson's (1915) normal growth curves for rats of the same age. Control rats on the "standard" food and yeast grew as well as those of Donaldson.

THE VITAMIN-B CONTENT OF THE WHOLE CORN KERNEL. *The varieties and food mixtures employed.* In view of the finding (Steenbock, 1919; Steenbock and Boutwell, 1920) that yellow corn is relatively rich in vitamin A, whereas white corn contains very little of this factor, the approximate concentration of vitamin B was studied in both yellow and white varieties of corn. The samples employed were a yellow dent Leaming variety, bought from a local seed store, and a white variety, furnished by the American Hominy Company, Indianapolis. All samples of corn fed were ground to a fine powder in a Nixtamal or Quaker City mill, so that the experimental animals were unable to pick out fragments of corn from the rest of the food mixture.

In order to have a basis for comparison of the different parts of the corn kernel as to their vitamin-B content, it was necessary to determine, first of all, the minimum amount of the entire kernel needed to supply sufficient vitamin B for normal growth of rats. A series of foods was prepared, analogous to the "standard" diet, but containing various percentages of ground whole corn; the corn replaced an equal weight of starch in the "standard" mixture in every case, and presumably the calorie value per gram of food was only slightly modified thereby. The foods thus provided a liberal supply of all known dietary essentials except vitamin B,

[2] Detailed records of body weights and food intake of animals used are given in the dissertation of H. M. Croll, Yale University, 1925.

which was the limiting factor in the adequacy of the rations for growth. The composition of this series of diets containing yellow corn follows:

Percentages

Casein	21	21	21	21	21	21	21	21	21
Starch	43	38	33	28	23	18	13	8	0
Corn kernel	5	10	15	20	25	30	35	40	48
Salt mixture	4	4	4	4	4	4	4	4	4
Lard	22	22	22	22	22	22	22	22	22
Cod liver oil	5	5	5	5	5	5	5	5	5

In the case of the white corn, only three different concentrations of the latter were fed,—20, 30 and 40 per cent of the food mixture. The smallest proportion of corn which promoted normal growth of rats was selected as the decisive amount for comparison with dissected yellow corn, or with commercial embryo of white corn.

Yellow corn. At least four rats were fed on each type of mixture. Most of those receiving 20 per cent or more of yellow corn grew at a normal rate (chart 1), and maintained a healthy condition. With 15 or 10 per cent of this corn in the diet, slower growth only was possible; and with 5 per cent, rats soon ceased to grow and then declined in weight. Therefore the minimum percentage of whole yellow corn, which furnishes an adequate amount of vitamin B for normal growth of rats from 40 to 140 grams, probably lies between 20 and 30 per cent of this corn in diets of the composition and calorie value described.

White corn. Foods containing 20, 30 and 40 per cent, respectively, of ground whole white corn were given to three or more rats. The animals on food containing 40 per cent of white corn (chart 4, rat 893; chart 6, rat 883) grew practically at a normal rate. Those receiving 30 per cent of corn in the diet showed equally good growth curves (chart 6, rats 916 and 917). Twenty per cent of whole white corn permitted growth at slightly less than the normal rate (chart 6, rats 1004 and 1005). The minimum admixture of this sample of white corn needed for normal growth of rats from 40 to 140 grams, lies between 20 and 30 per cent of a food of the type used, as in the case of the yellow corn studied.

McCollum, Simmonds and Pitz (1916–17) and Osborne and Mendel (1919) have shown that 15 or 20 per cent of *whole wheat* in the diet supplies adequate vitamin B for normal growth of young rats. It is thus evident that *yellow and white whole corn do not differ greatly from whole wheat, in their content of vitamin B.*

THE DISTRIBUTION OF VITAMIN B IN THE CORN KERNEL. *Hand-dissection of yellow corn.* A study of the anatomy of the corn kernel shows that by making a U-shaped cut into the dry grain, it is possible to separate all of the embryo from a large part of the endosperm and bran. A special

Fig. 2

Fig. 3

Fig. 2. Illustrating the separation of the embryo from the endosperm of the maize kernel by means of dental forceps.

Fig. 3. Illustrating the products obtained by dissection of the maize kernel: *A*, the entire maize kernel; *B*, endosperm 1; *C*, embryo 1; *D*, endosperm 2; *E*, embryo 2; *F*, bran 3; *G*, endosperm 3; *H*, embryo 3; *I*, bran 4; *J*, endosperm 4; *K*, embryo 4; *L*, bran 5; *M*, endosperm and embryo 5.

type of dental forceps was a very convenient instrument for making this dissection in the manner shown in the accompanying illustration (fig. 2). The cut surfaces of the endosperm were carefully examined to insure complete separation of the embryo. Figure 3 illustrates the application of the procedure. In three samples separated according to this method, the following yields were obtained:

FRACTION OF KERNEL	PROPORTIONS OF WHOLE CORN KERNEL CONTAINED IN DISSECTED PARTS		
	per cent	*per cent*	*per cent*
Embryo 1 (including some endosperm and bran).....	42.5	45.3	44.3
Endosperm 1 (including most of the bran).....	57.5	54.7	55.7

In separations by this procedure the embryo was mixed with a relatively large amount of endosperm, and a small amount of bran, the endosperm portion containing the rest of the bran. Therefore, in order to effect a more complete separation a second method was used. The first U-shaped

cut into the dry kernel was made, as before, with the dental forceps, and the endosperm and bran fractions, entirely free from embryo, were removed. A second, longitudinal cut was made into the wedge-shaped piece, at right-angles to the first, between the two flat surfaces of the kernel. The front portions, retaining all of the embryo, a smaller amount of endosperm, and a trace of bran, constituted embryo 2; the back parts, containing only endosperm and bran, added to the original endosperm and bran portions, constituted endosperm 2. The comparative weights of these fractions showed that a more complete removal of endosperm and bran from the embryo portion had been accomplished (fig. 3). In four dissections by this method, the following proportions were found:

FRACTION OF KERNEL	PROPORTIONS OF WHOLE CORN KERNEL CONTAINED IN DISSECTED PARTS			
	per cent	*per cent*	*per cent*	*per cent*
Embryo 2 (including small amount of endosperm and bran)	27.5	29.2	24.7	28.5
Endosperm 2 (including most of the bran)	72.5	70.8	75.3	71.5

Both of the preceding methods of dissection yielded information about endosperm and bran, entirely free from embryo. On the other hand, the embryo portions were not entirely free from endosperm or bran, nor was the bran obtained pure. Consequently in a third mode of dissection the whole corn was put into boiling water, the heat was then turned off, and the corn was allowed to remain in the hot water for three minutes, after which it was soaked in cold water for $\frac{3}{4}$ to $1\frac{1}{2}$ hours. The softened corn could then be dissected easily, so as to separate the parts more completely. The bran (hull and pedicel or "tip-cap") was peeled off from the softened grain with a scalpel. The embryo was next removed by wedging the dull edge of the scalpel between the surfaces of the embryo and endosperm, and lifting the germ out with this instrument. As a rule the surface of the embryo, when removed, had a smooth, waxy appearance, and very little, if any, endosperm tended to adhere. Any attached flakes of endosperm were scraped off as completely as possible, special care being taken that no embryo was left with the endosperm fraction. The three portions were dried for 12 to 15 hours in a warm room, to the original weight of the whole corn taken, before percentage calculations were made. Dissection in this way showed the following proportions of fractions:

FRACTION OF KERNEL	PROPORTIONS OF WHOLE CORN KERNEL CONTAINED IN DISSECTED PARTS		
	per cent	*per cent*	*per cent*
Embryo 3	12.88	13.1	13.1
Endosperm 3	87.12	80.1	80.7
Bran 3		6.8	6.2

This method has the advantage of accomplishing more complete removal of endosperm from the bran and embryo, and it permits a more accurate calculation of the proportions of these three structural parts in the corn kernel, than do the previous dissections. However, it has the disadvantage of requiring a soaking of the grain in water, and thereby introducing the possibility of slight diffusion of vitamin B from one part of the grain to another, during the soaking. (Nevertheless, the results of feeding the parts thus separated do not suggest that any such diffusion occurred.)

In order to lessen the possibility of any diffusion of vitamin B because of exposure of the corn to moisture, a fourth procedure also was adopted. A few grains of corn at a time were placed in a wire sieve over a vessel of boiling water, so that they were exposed to the steam. Five to 10 minutes of this steaming process moistened and softened the grain sufficiently to make possible the removal of the bran and embryo with a scalpel, as in the preceding method; in this case it was slightly more difficult to effect such removal, since the corn was not so thoroughly moistened within the kernel. After the three fractions were dried in air to the original weight of corn taken, as in the third procedure, the following distribution was found:

FRACTION OF KERNEL	PROPORTIONS OF WHOLE CORN KERNEL CONTAINED IN DISSECTED PARTS
	per cent
Embryo 4	13.6
Endosperm 4	79.2
Bran 4	7.2

Finally, in order to obtain a quantity of the bran, corn kernels were soaked in warm water for 3 to 15 minutes, after which the bran was easily removed from the rest of the grain with a scalpel. The proportions of the air-dry parts were as follows:

FRACTION OF KERNEL	PROPORTIONS OF WHOLE CORN KERNEL CONTAINED IN DISSECTED PARTS		
	per cent	*per cent*	*per cent*
Embryo and endosperm 5	94.0	93.8	94.2
Bran 5	6.0	6.2	5.8

The methods just described made available fractions of the embryo, endosperm and bran of yellow corn, representing the main structural divisions of the grain. These portions were ground to a fine powder, as in the case of the whole corn kernel, and were used as possible sources of vitamin B, to determine whether it is distributed throughout the grain, or localized in certain portions thereof.

The embryo of yellow corn. Corn embryo incorporated into paste foods. Powdered embryo, prepared in the four ways just described, was tested on rats. If all of the vitamin B present in the whole kernel is confined

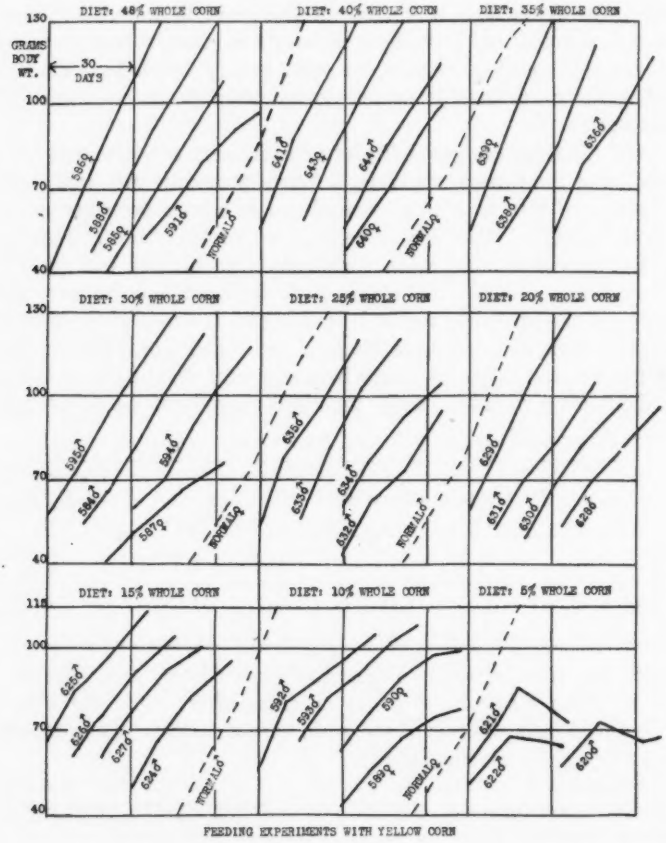

Chart 1. Changes in body weight of rats on otherwise adequate diets, in which various proportions of whole yellow corn furnished the vitamin B of the ration. Twenty to thirty per cent of yellow corn in these diets furnished sufficient vitamin B for normal growth of young rats during the period selected.

to the embryo, foods with a content of embryo equivalent to that present in mixtures containing 10, 15, 20 and 30 per cent, respectively, of whole corn, should permit growth comparable to that obtained when these percentages of whole corn are fed. Thirty per cent of whole yellow corn

in our "standard" mixtures supplied an adequate amount of vitamin B for normal growth. Foods were prepared in which the different embryo samples were present in quantities calculated to be equivalent to their representation in the whole corn used. Thus embryo 1, separated by the first method of dissection, in one preparation represents 42.5 per cent of the entire corn kernel. Therefore, a food containing 12.7 per cent (30 × 42.5 per cent) of embryo 1, is equivalent to the amount present in food containing 30 per cent of the whole corn. Such a mixture was tested on four rats, control rats being kept at the same time on food containing 30 per cent of whole corn as a source of vitamin B. The animals on a diet including 12.7 per cent of embryo 1 (chart 2, rats 834, 835, 836; chart 4, rats 838 and 839) grew almost as well as those on a ration of 30 per cent of whole corn (chart 2, rats 808, 809, 810; chart 4, rat 837). Corn embryo prepared by the other modes of dissection was studied in the same way, through comparison of growth on various percentages of the entire corn kernel, and on foods containing embryo equivalent to that in the diets including whole corn. Some of the rats (chart 2) receiving embryo equivalent to the corresponding amount of the whole kernel grew almost as well as those on the corresponding content of whole corn; others gained equally as well, and one rat on food containing 2.62 per cent of embryo 3 (rat 1013) made greater gains in body weight than the animal on the equivalent quantity (20 per cent) of the whole corn kernel (rat 1052). From these findings it appears that *almost, if not quite, all of the vitamin B present in the yellow corn kernel, is contained in the embryo.*

Comparison of whole corn, embryo and endosperm, fed as measured supplements to the "standard" food. The relative concentration of vitamin B in the kernel as a whole, in the embryo, and in the endosperm (+ bran), was studied, by feeding equal quantities of them, as supplements to the "standard" ration, after rats had declined in weight on the "standard" food alone, because of vitamin-B deficiency (chart 3). With both "standard" food 1 and "standard" food H, a daily supplement of one gram (and later 1.5 gram) of embryo 1 (rat 832) produced distinctly better growth than the same amounts of the whole corn kernel (rat 830). The decline in weight of rats 847 and 800 on the "standard" diet was not greatly checked by feeding one gram daily of endosperm (+ bran) 1, and the rats ultimately died. These experiments indicate that *the vitamin B of the corn kernel is concentrated largely in or around the embryo, and that the endosperm and bran fraction contains relatively little, if any, of this vitamin.*

Comparison of whole yellow corn and embryo fed as measured supplements to the "standard" food. Another method of determining whether the embryo contains all of the vitamin B was employed. Rats were fed the "standard" diet devoid of vitamin B until they ceased growing

and began to lose weight. Then two rats of the same litter, of the same sex, and of similar weight were used for comparison in each case. One of each pair was fed a daily weighed amount of whole corn, apart from the "standard" food, and the second rat was given a weighed quantity

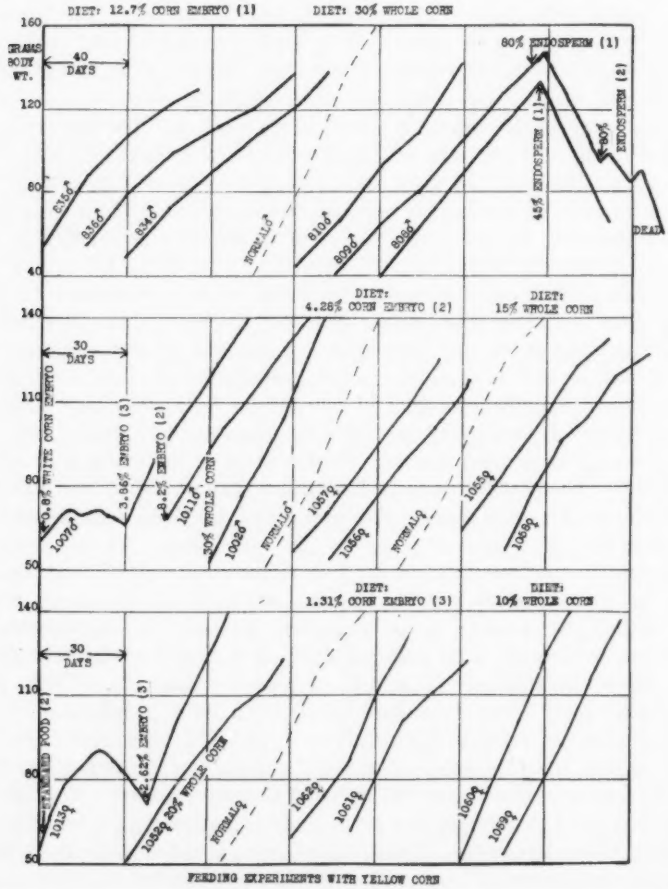

Chart 2. Changes in body weight of rats on otherwise adequate diets, in which whole yellow corn or equivalent amounts of the embryo of yellow corn constituted the sole source of vitamin B. Thirty per cent of whole corn, or its equivalent (12.7 per cent) of embryo 1, were of essentially equal vitamin-B potency. Similar results are shown for other comparisons of equivalent percentages of whole corn and embryo. Replacement of whole corn by the embryo-free endosperm (rats 808 and 809) resulted in prompt decline.

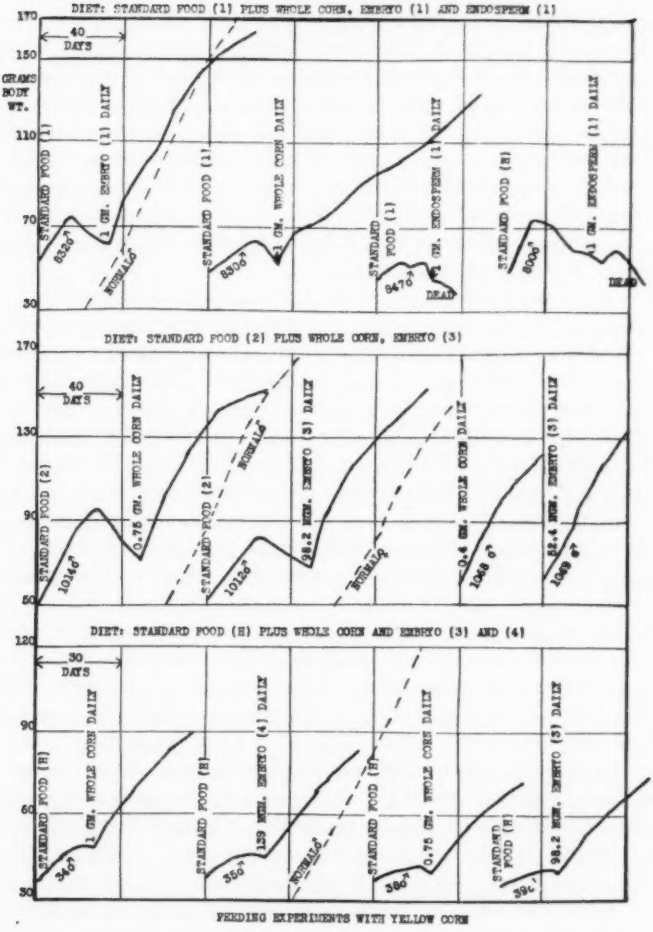

Chart 3. Changes in body weight of rats receiving the "standard" food, with measured daily allowances of whole yellow corn, corn embryo or endosperm, as possible sources of vitamin B. One gram of embryo 1 was superior in vitamin-B potency to the same amount (1 gram) of whole corn, whereas one gram of endosperm 1 did not permit any growth whatever. One gram of whole corn (rat 34) or its equivalent (139 mgm.) of embryo 4 (rat 35) showed essentially equal vitamin-B potency. Similar comparisons of other equivalent amounts of whole corn and embryo are shown.

of dissected corn embryo equal to that calculated to be present in the whole corn offered to the first animal (chart 3). Rats receiving a daily allowance of embryo 3 or 4 equivalent to the amounts of the entire kernel fed, grew as well as, or even slightly better than the rats which were given the whole kernel. In all cases the growth curves of the pairs of animals were similar. Normal growth occurred to about the same point, and there was retardation at the end to about the same extent. It appears, then, that there was as much vitamin B in the embryos as in the corresponding amounts of whole corn fed. This constitutes rather strong evidence for the conclusion that *practically all of the vitamin B in the corn kernel is contained in the embryo.*

The endosperm and bran of yellow corn. Corn endosperm incorporated into paste and powder foods. For experiments on corn endosperm, the ground fractions of endosperm (+ bran) 1 and 2, and of endosperm 3 and 4 alone, prepared in four methods of dissection, were used. Since incorporation of 30 per cent of the entire corn kernel into the "standard" food had been found to supply sufficient vitamin B to permit normal growth, food was made up to contain 30 per cent of endosperm (+ bran) 1. If vitamin B is distributed evenly throughout the kernel, and if the concentration of vitamin B in the endosperm and bran fraction is as great as it is in the whole grain, rats should grow normally on food containing 30 per cent of endosperm and bran. However, inspection of chart 4 shows that rats not only did not grow normally, but they soon declined in weight, when fed a ration containing 30 per cent of endosperm 1 as a possible source of vitamin B (rats 837, 838, 839 and 840). Their food intake decreased, their coats became rough, they scattered food and became restless, and they presented the general appearance of poorly nourished animals. Rats were able to grow on corn embryo or whole corn, after failing to grow on corn endosperm. It is evident that *the endosperm and bran fraction of the corn kernel is deficient in vitamin B.*

Foods containing even higher percentages of endosperm were fed to rats to determine whether there was any vitamin B whatever in this fraction. Rats fed 45 per cent of endosperm (+ bran) 1 (chart 4, rats 893 and 895) soon ceased growing. Rat 808 (chart 2) which had grown normally to 136 grams body weight, on a ration of 30 per cent of whole corn, when changed to a diet containing 45 per cent of endosperm (+ bran) 1, began to decline and continued to lose weight until the diet was changed. Replacement of 45 per cent of starch in the "standard" mixture with corn endosperm as a possible source of vitamin B, produces a food inadequate for growth.

In order to incorporate more corn endosperm into the diet, without decreasing the supply of adequate protein, inorganic salts, and vitamin A, it was necessary to make up a powder food on a different basis than the

original "standard" paste food. Accordingly, a mixture containing 80 per cent of endosperm of corn, 17 per cent of casein, and 3 per cent of the Osborne and Mendel salt mixture, was fed to rats, with an additional allowance of 0.5 cc. of cod liver oil daily. Even with this large inclusion of endosperm in the liberal food intake of 9 to 11 grams per day in the beginning, there was not enough vitamin B present to permit growth; in fact, all rats on this food declined rapidly in weight (chart 4, rats 1 and 6), and died unless the diet was changed (chart 2, rat 809; chart 4, rats 837, 893 and 895).

Control experiments were performed with a food containing 80 per cent of corn starch, 17 per cent of casein, and 3 per cent of salt mixture, with a daily vitamin supplement of 0.5 cc. cod liver oil and 100 mgm. dried brewery yeast; rat 15 (chart 4) grew at a normal rate on this diet, showing that it is adequate in all essential dietary factors. When yeast was omitted (chart 4, rats 10 and 15), animals declined in weight, showing that the food mixture actually was inadequate in vitamin B. Again it is apparent that *the vitamin-B content of corn endosperm (+ bran) is very low indeed, and probably so small as to be of no practical significance.*

That the food mixture containing 80 per cent of corn endosperm was satisfactory in every respect except in vitamin B, and that its failure to produce growth was not attributable to a toxic material in the corn, has been proved by the experiment on rat 6 (chart 4); this animal failed to grow on the diet until yeast was added, whereupon it gained at a normal rate. No evidence for the presence of a toxic substance in *corn* was found in any of our experiments. This is of interest, since Hart, Miller and McCollum (1916) and McCollum, Simmonds and Pitz (1916) believed that they found in *wheat* a substance toxic to swine and rats. However, Hart, Steenbock, Humphrey and Hulce (1924–25), from data accumulated since the factors of nutrition have been more perfectly understood, concluded that "it does not seem necessary to assume the presence of an inherent toxic factor in the wheat grain in explanation of the nutritive failure following the use of a ration made wholly from the wheat plant." On the other hand, Hoffman (1925) has offered evidence of the toxicity of wheat germ oil.

The bran of yellow corn. As the fractions of endosperm 1 and 2 of yellow corn, which included most of the bran, were shown to be deficient in vitamin B, obviously the pure bran cannot be rich in this factor. Foods containing 80 per cent of endosperm 1 or 2, which are calculated to include at least 5.6 per cent of bran, were unable to produce any growth at all in rats. Much smaller percentages of embryo 2 and 3 were shown to be entirely adequate. It is apparent by contrasting the experiments on embryo with those on endosperm (+ bran), that there cannot be a high concentration of vitamin B in the isolated bran. An attempt was made

to find out whether corn bran contains any vitamin B whatever. Rats which were fed the "standard" food H and a daily allowance of 0.5 gram of bran 5, after they had declined in weight on the "standard" diet alone were unable to grow, soon lost weight, became very weak and unsteady in gait, and showed increased susceptibility to infections (chart 5, rats

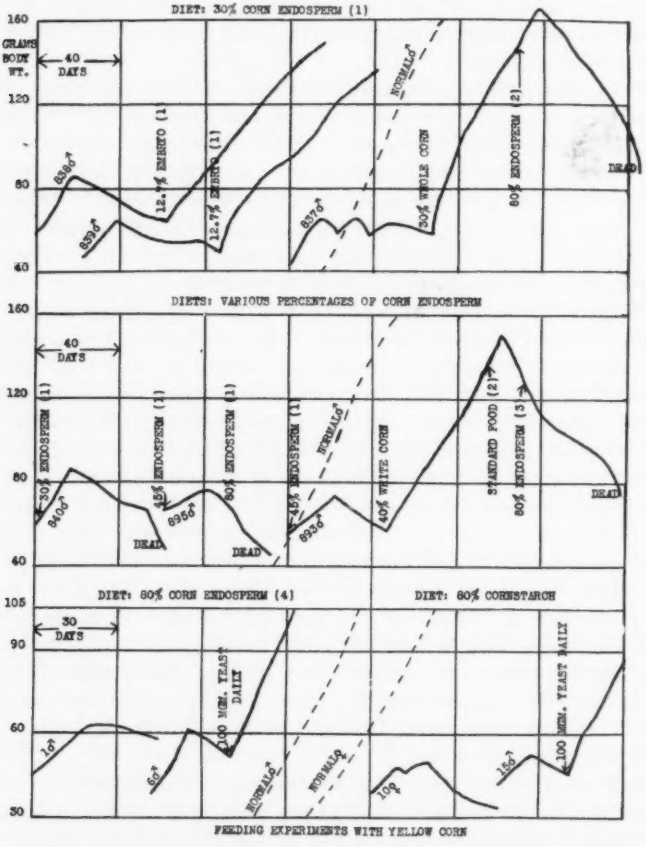

Chart 4. Changes in body weight of rats on otherwise adequate diets, in which various proportions of yellow corn endosperm were incorporated, as possible sources of vitamin B. Thirty, 45, and even 80 per cent of corn endosperm did not permit any growth; in fact, rats promptly declined on these rations. Normal growth on a diet (rat 6) containing 80 per cent of endosperm 4, supplemented with a daily allowance of 100 mgm. of dried yeast, proved that the failure of the ration without yeast was due to deficiency in vitamin B, and not to any toxicity of the corn.

538 and 539). Rats given a daily supplementary feeding of one gram[3] of bran 5 did not grow, but maintained their body weight (chart 5, rats 533 and 537). Both appeared very badly nourished, and one of them (rat 537) developed an ear infection. The other rat, after failing to grow on the "standard" diet plus corn bran, grew rapidly when 100 mgm. of yeast were added daily to the ration. This proves that the failure of the bran to promote growth was not attributable to any toxic substance in the bran, but due to its deficiency in vitamin B. The *concentration* of vitamin B is thus shown to be much smaller in the bran of the *corn* kernel than in the kernel as a whole, a condition quite at variance with the distribution in the *wheat* kernel. These experiments suggest strongly that *the vitamin-B content of corn bran is too low to be of practical significance.*

The embryo (commercial) of white corn. It was desired to obtain some idea of the amount of vitamin B in commercial corn germ and, by com-

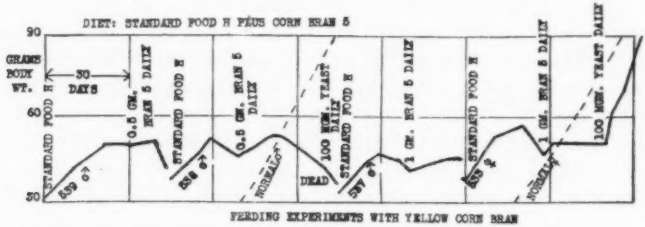

Chart 5. Changes in body weight of rats receiving the "standard" food, with measured daily allowances of yellow corn bran, as possible sources of vitamin B. One-half gram, or even one gram of bran 5 did not permit growth. Failure of the ration to support growth was due to vitamin-B deficiency and was not attributable to the presence of a toxic substance in the bran, since growth occurred when the ration was further supplemented by 100 mgm. of dried yeast daily (rat 533).

paring with whole corn, to determine whether the germ as separated in the industrial hominy mill contains all or most of the vitamin B present in the kernel. The whole white corn and commercial corn germ were taken from the same sample of grain, both being furnished by the American Hominy Company of Indianapolis. The method of removal of the germs as described by F. C. Atkinson, Chief Chemist, is given below:

> The corn germs are separated purely by mechanical means, only a little moisture being added to the corn preparatory to grinding it for removal of the germs, which occurs within 10 minutes after the corn enters the mill. As the corn enters, a small stream of water, amounting to about 5 per cent of the total weight of the corn, flows into the stream of corn, and is allowed to travel in the conveyor, while the corn is thoroughly agitated, whereby the moisture is permitted to penetrate the hull of the

[3] The moistened corn bran was eaten less readily than whole corn and corn embryo. However, over 95 per cent of the bran offered was consumed.

corn and loosen the germs somewhat from the starchy portion of the kernel. It then passes through an attrition grinder, where it is broken open and the germs are removed by abrasion. In this case the germs were air dried for two or three hours in a warm dry room, in order to obviate subjecting them to the temperature to which they might be exposed for a few minutes in the mill. These germs constitute about 4 per cent of the original corn. It is impracticable to make a complete separation by mechanical means, and the germs are badly contaminated with the starchy portion of the corn.

Such commercial embryo of white corn, ground to a fine powder, was tested as a possible source of vitamin B.

Embryo (commercial) of white corn incorporated into paste foods. Since our experiments on yellow corn had shown that most, if not all, of the water-soluble vitamin B is contained in the embryo, an attempt was made to ascertain whether the commercial corn germ contains all of the vitamin B in white corn. It was shown earlier that 20 per cent of whole white corn in the diet produces growth at slightly less than the normal rate. A comparison was made between growth on this food (chart 6, rats 1005 and 1004), and on a diet containing an equivalent amount (0.8 per cent) of white corn germ (based on the estimate that this germ represents 4 per cent of the whole grain) (chart 2, rat 1007; chart 6, rats 838 and 1006). On the latter diet, growth did not occur. Food with 1.4 per cent of embryo of white corn (equivalent to the amount of embryo in a diet containing 20 per cent of the white corn kernel, based on an estimate[4] that corn germ constitutes 7 per cent of the kernel) permitted more satisfactory growth (chart 6, rats 1008 and 1009), than food with 0.8 per cent of embryo; but not such rapid growth as did a mixture with 20 per cent of whole corn incorporated therein.

Embryo (commercial) of white corn fed as measured supplement to the "standard" food. Further study was made of the vitamin-B content of commercial corn germ. Calculations were made of the amounts of embryo present in a day's ration of foods containing 20, 30 and 40 per cent, respectively, of whole white corn (based on a daily food intake of 6 grams,[5] and on the estimate that this commercial corn germ constitutes 4 per cent of the whole grain). The estimates showed that there was a daily consumption of 48, 72 and 96 mgm., respectively, of the embryo in these foods. Rats were therefore fed with the "standard" food mixture, supplemented with these amounts of commercial (white corn) embryo, and the growth was compared with that of rats receiving 20, 30 or 40 per cent of whole corn in their rations (chart 6). In every case, the growth on this commercial corn germ was decidedly slower than that induced by the corresponding percentage of the entire white corn kernel.

[4] Estimate of the Corn Products Refining Company, Edgewater, N. J.

[5] This was slightly low, as control rats growing normally to 140 grams consumed 7 to 9 grams of the "standard" food daily.

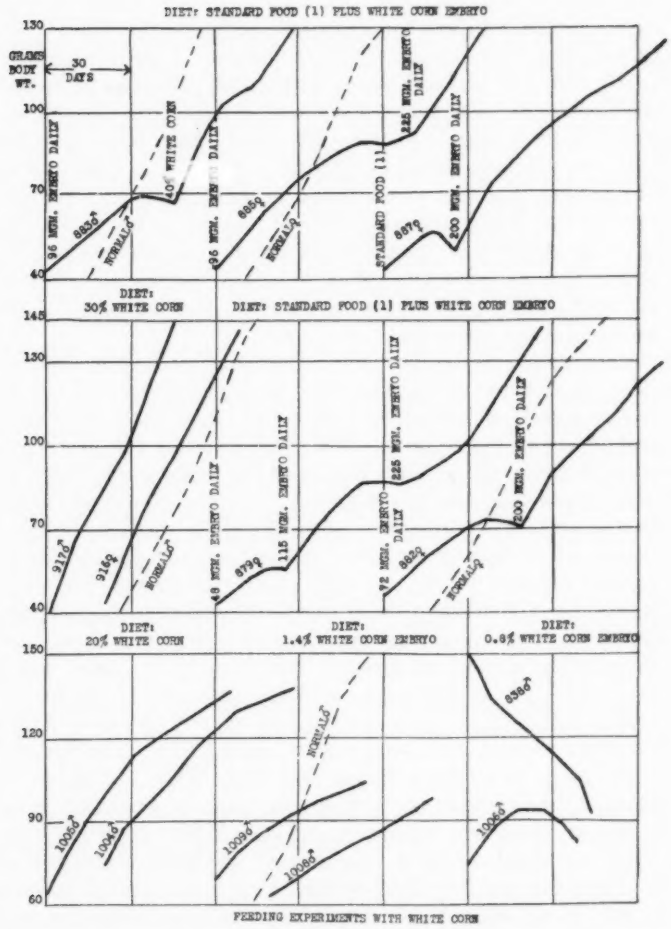
DIET: STANDARD FOOD (1) PLUS WHITE CORN EMBRYO
GRAMS BODY WT.
30 DAYS
96 MGM. EMBRYO DAILY
40% WHITE CORN
NORMALS
96 MGM. EMBRYO DAILY
STANDARD FOOD (1)
225 MGM. EMBRYO DAILY
200 MGM. EMBRYO DAILY
DIET: 30% WHITE CORN
DIET: STANDARD FOOD (1) PLUS WHITE CORN EMBRYO
48 MGM. EMBRYO DAILY
115 MGM. EMBRYO DAILY
92 MGM. EMBRYO DAILY
225 MGM. EMBRYO DAILY
200 MGM. EMBRYO DAILY
DIET: 20% WHITE CORN
DIET: 1.4% WHITE CORN EMBRYO
DIET: 0.8% WHITE CORN EMBRYO
FEEDING EXPERIMENTS WITH WHITE CORN

Chart 6. Changes in body weight of rats on otherwise adequate diets, in which whole white corn or equivalent amounts of commercial embryo of white corn, constituted the sole source of vitamin B. Twenty to 30 per cent of the white corn kernel in these diets furnished sufficient vitamin B for normal growth of young rats during the period selected (rats 916, 917, 1004 and 1005). Twenty per cent of whole corn was distinctly superior in vitamin-B potency to equivalent amounts (0.8–1.4 per cent) of commercial embryo (rats 1004, 1005, 1008, 1009, 1006, 838). Daily allowances of 225 mgm. of commercial embryo (rats 885 and 879) fed as supplements to the "standard" food, permitted normal growth to 140 grams body weight.

Finally, an attempt was made to determine how much commercial corn germ must be fed daily, as a supplement to the "standard" diet, to produce normal growth up to 140 to 150 grams. Daily feedings of 48, 72, 96, 115, 150, 200 and 225 mgm., respectively, of white corn embryo were tried (chart 6). On amounts below 225 mgm. per day, growth at a normal rate soon ceased, whereas 225 mgm. daily facilitated normal growth of rats weighing 100 to 140 grams. Probably a daily allowance of 225 to 250 mgm. of this commercial corn germ is adequate as the sole source of vitamin B, for normal growth of rats up to 140 to 150 grams. Certainly this amount is greatly in excess of the amounts of embryo calculated above as being present in the daily rations of rats growing normally up to 140 to 150 grams on the foods that included 30 and 40 per cent of whole white corn.

The interpretation of these experiments is not clear: either the embryo in white corn does not contain so large a proportion of the vitamin B of the entire kernel, as does yellow corn embryo; or vitamin B is partially destroyed in the milling process, thus accounting for the lower content of vitamin B in the commercial embryo; or the germ, as separated in the industrial hominy mill, does not represent the entire embryo of the kernel, some having been lost in the process of milling. The method of separation of the germs in the hominy mill does not suggest any destruction of the water-soluble vitamin B; on the other hand, mechanical loss of some of the germ, and distribution to other parts of the kernel, appears possible. The low estimates of 4 and 7 per cent, respectively, given by two milling companies as the content of germ in the whole grain, when compared with 10 to 13 per cent found by hand-dissection (Hopkins, Smith and East, 1903; Osborne and Mendel, 1914; and Wagner, Sherman, 1924), tend to support this possibility of milling loss. The results of a comparison made by Hopkins, Smith and East (1903), of the chemical composition of the parts of the corn kernel, when separated by hand and by the hominy mill, also constitute evidence that certain milling processes may not effect complete separation of the structural parts of the kernel. Since the portions of the white corn kernel tested for vitamin B were not dissected by hand, a conclusive answer cannot be made to the question of the distribution of this vitamin in the white corn kernel. However, there is no reason to expect that the distribution of vitamin B throughout the kernel differs greatly in yellow and white varieties of corn.

SUMMARY AND CONCLUSIONS

Earlier investigations have shown that maize, like the other cereal grains, contains an abundance of vitamin B and has antineuritic properties. The distribution of vitamins within the whole grains and in their

milling products has not yet been adequately ascertained. Various earlier studies on the antineuritic vitamin of rice, wheat and maize emphasized the peripheral layers and the embryo of these seeds, as the site of greatest concentration of this substance, the endosperm being considered deficient in this factor.

The unexpected discovery that vitamin B is not confined to any one part of the *wheat* kernel, but is distributed throughout the entire grain, has called for comparative investigations of other cereals. In the present study an attempt was made to determine the mode of distribution of vitamin B in the structural parts of the *maize* kernel.

It was demonstrated that between twenty and thirty per cent of either yellow or white whole maize incorporated in otherwise adequate diets of the "standard" type selected, furnishes an adequate supply of vitamin B for normal growth of young albino rats from 40 to 140 grams body weight.

Methods were devised for separation of the maize kernel into its structural parts. Feeding experiments with portions of the yellow maize kernel showed that *practically all of the vitamin B of the whole grain is found in the embryo. The endosperm contains practically no vitamin B, while the bran includes an extremely small amount, if any, of this factor.*

Experiments with commercially-milled embryo of white corn indicated the possibility of some loss of vitamin B incident to its production. Tentative explanations are proposed.

No evidence was secured for the presence of a toxic substance in maize.

From investigations still in progress it appears that rice and wheat may be similar, in carrying vitamin B distributed throughout the kernel, while a different allocation is found in maize. Evidently general statements regarding the uniformly similar distribution of vitamin B in the kernels of all of the cereals are not justified; each cereal grain should be considered separately.[6]

Grateful acknowledgment is extended to Prof. Arthur H. Smith, for helpful advice during the progress of the work.

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[6] We have been informed of unpublished experiments conducted by Dr. M. S. Fine, Postum Cereal Company, Battle Creek, Michigan, which have yielded results similar to those reported here.

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THE EFFECT OF INSULIN ON THE MORPHOLOGICAL BLOOD PICTURE, WITH A NOTE ON THE RELATION OF DIET TO THE CONVULSIONS INDUCED BY INSULIN[1]

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INSULIN AND THE MORPHOLOGICAL BLOOD PICTURE. That insulin affects both the chemical and morphological blood picture has been observed by several investigators. Drabkin and Edwards (1) reported an increase in the hemoglobin content. Best and Ridout (2) observed in a number of cases that a fall in blood sugar was accompanied by a rise in hemoglobin concentration in both normal and diabetic animals. Olmsted and Taylor (3) found that the hemoglobin concentration rose sharply two or three hours after insulin injection in decerebrate and decapitate cats. Rabbits on the contrary usually showed a slight fall. Nitzescu and Mangiuca (4) demonstrated a leucopenia followed by leucocytosis, while Mazzocco and Morera (5) found no modification in the number of red or white cells, or in the hemoglobin count.

Drabkin and Edwards postulated from the increase in hemoglobin content that the injection of insulin produced a condition of anhydremia. Clinically the concentrating of the blood manifests itself in a greater water output through the skin. Fletcher and Campbell (6) report profuse sweating. Villa (7) observed that the administration of 50 units of insulin to a diabetic patient at the end of two hours reduced the blood volume by 11.6 per cent, while in a normal person 40 units of insulin brought forth after one hour a reduction of 9.8 per cent in blood volume.

Very recently, however, Hamilton, Barbour and Warner (8) have cast doubt upon the existence of anhydremia as a result of hypoglycemia due to insulin, for they have found that convulsive doses of pancreatic hormone do not alter the concentration of the blood as determined by either total solid content or the specific gravity. Haldane, Kay and Smith (9) also have reported that the administration of insulin to normal rabbits leads to an appreciable rise in blood volume. In a more recent publication Kay and Smith (10) state that different preparations of insulin produce diverse

[1] Part of this paper appeared in abstract in the Proceedings of the Society for Experimental Biology and Medicine, 1924, xxii, 169.

effects. They claim that subcutaneous injection of insulin hydrochloride, now on the English market and prepared by Dodd's process, brings about a considerably smaller increase in blood volume than that produced by the cruder kinds used in the earlier experiments. It may no doubt be true that the preparations on the market differ in chemical composition and may on that account give discordant results.

We have studied the blood picture in rabbits after insulin treatment and have found that the injection of this hormone produces, as the tables show, a rise in the number of erythrocytes per cubic millimeter and also in the number of leucocytes. The fact that both an erythrocytosis and a leucocytosis exist simultaneously is strongly suggestive of the existence of anhydremia. This condition Drabkin and Edwards have postulated from the increased hemoglobin content following subcutaneous administration in dogs. The increase in the red and in the white cells argues more favorably for the existence of a state of anhydremia than the increase in hemoglobin or in the red cells alone.

Nitzescu and Mangiuca have reported the occurrence under the influence of insulin of a leucopenia followed by a leucocytosis. In our experiments and under the conditions with which we have worked, no leucopenia was ever encountered.

The per cent increase in the white was always greater than in the red count. From this observation we believe that the mild leucocytosis is partly due to increase in the blood concentration and partly due to the presence of protein and other substances in the insulin preparation. The rise in the number of cell components of the blood was proportional to the fall in blood sugar. The greater the fall in the sugar content of the blood, the greater was the anhydremia. There was apparently no relation between the size of the insulin dose and the degree of anhydremia. As the anhydremia progressed, the blood drawn increases in viscosity, and sometimes difficulty was experienced in obtaining for analysis the desired quantity of blood from the ear. This difficulty was probably not due to an increased tendency of the blood to clot, for J. La Barre (11) found that American insulin does not affect coagulability.

We have made differential counts, of which we append but two, and find no appreciable changes in the relative distribution of the various types of white cells.

DIET IN RELATION TO INSULIN CONVULSIONS. In reference to convulsions resulting from insulin, there seems to be, judging from the later discussions in the literature, no regularity in their onset. Olmsted and Taylor, for instance, report that there is a wide variation in rabbits. Some will not develop convulsions at all even with ten times the normal dose of insulin, while others develop convulsions within half an hour after the injection of small amounts. From a clinical standpoint, the toxic

Effect of Insulin on the Morphological Blood Picture*

SERIES I

Diet: Oats and fresh cabbage

Experiment 1. Weight of rabbit, 2200 grams. Insulin injected, 3 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.105	6,900,000	7,375
Time after injection:			
1 hour 10 minutes	0.075	6,936,000	10,575

Remarks: No convulsions.

Experiment 2. Weight of rabbit, 1640 grams. Insulin injected, 3 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.098	6,720,000	5,700
Time after injection:			
1 hour 20 minutes	0.066	6,890,000	9,276
2 hours 55 minutes	0.035	7,480,000	9,924

Remarks: No convulsions.

Experiment 3. Weight of rabbit, 2300 grams. Insulin injected, 3 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.136	6,632,000	4,332
Time after injection:			
1 hour 20 minutes	0.104	7,672,000	4,700
2 hours 25 minutes	0.066	6,608,000	10,200
4 hours 55 minutes	0.079	6,464,000	8,425

Remarks: No convulsions.

Experiment 4. Weight of rabbit, 2200 grams. Insulin injected, 4.5 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.108	6,600,000	6,750
Time after injection:			
1 hour 15 minutes	0.058	7,200,000	8,000
2 hours 30 minutes	0.048	7,800,000	8,300

Remarks: No convulsions.

Experiment 5. Weight of rabbit, 2000 grams. Insulin injected, 15 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.100	6,600,000	6,750
Time after injection:			
1 hour 15 minutes	0.079	7,200,000	8,000
2 hours 30 minutes	0.048	7,800,000	8,300

Remarks: No convulsions.

*The insulin used in all the experiments recorded in the paper came from the Eli Lilly Co.

Experiment 6. Weight of rabbit, 2100 grams. Insulin injected, 40 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.110	4,808,000	11,740
Time after injection:			
1 hour 35 minutes	0.079	5,440,000	14,124
3 hours	0.048	5,565,000	19,800

Remarks: No convulsions.

Experiment 7. Weight of rabbit, 2200 grams. Insulin injected, 6 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
Normal		7,520,000	7,500
Time after injection:			
1 hour 20 minutes		7,160,000	7,800
2 hours 25 minutes		7,784,000	10,340

Remarks: No convulsions.

	DIFFERENTIAL WHITE COUNT					
	Polymorphonuclear leucocytes	Lymphocytes, large	Lymphocytes, small	Eosinophiles	Basophiles	Neutrophiles
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Normal	50.0	12.0	33.0	4.5	0.5	
Time after injection:						
1 hour 20 minutes	37.5	15.0	41.0	5.5	0.5	0.5
2 hours 30 minutes	43.0	17.0	33.0	7.0		

Experiment 8. Weight of rabbit, 2100 grams. Insulin injected, 40 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.110	4,808,000	11,740
Time after injection:			
1 hour 35 minutes	0.079	5,440,000	14,124
3 hours	0.048	5,656,000	19,800

Remarks: No convulsions.

	DIFFERENTIAL WHITE COUNT					
	Polymorpho-nuclear	Lymphocytes, large	Lymphocytes, small	Eosinophiles	Basophiles	Neutrophiles
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Normal	56	10	30	7		
Time after injection:						
1 hour 35 minutes	50	15	34	2		
3 hours	54	14	29	2	1	

effects of insulin and its power to utilize sugar seem also to show great variation.

An interesting fact brought out by our experiments is the dependency upon diet of the convulsions associated with insulin. This relation has also been observed by others. McCormick, Macleod, Noble and O'Brien (12) found that animals with large amounts of glycogen in their tissues were more resistant to insulin than were animals poor in glycogen. Brugsch, Benatt, Horster and Katz (13) also explained the convulsions on the basis of cerebral sugar starvation. On the other hand, C. F. Cori and G. T. Cori (14) have found that the free sugar in the brain of starving rats and mice is not influenced by insulin. These two investigators think it improbable that the hypoglycemic symptoms after insulin injection are due to the lowering of the glucose tension in the brain centers. Other workers, also, could make no correlation between glycogen content and excitability to convulsions. Blatherwick, Long, Bell, Maxwell and Hill (15) fed rabbits alfalfa hay alone or alfalfa hay and crushed barley. The former diet is base-forming and carbohydrate-poor, while the latter is acid-forming and rich in carbohydrate. They found convulsions much easier to produce in rabbits fed on alfalfa hay than those fed on alfalfa and barley. The greater resistance on the latter diet does not appear to be due to an increase in the glycogen content of the tissues, since animals which were maintained on both diets and fasted twenty-four hours yielded practically the same amount of glycogen in liver and muscle. Winter and Smith (16) also found in the tissues of rabbits and rats, recovered from insulin convulsions by means of adrenalin, more glycogen than at the convulsion level. Furthermore they observed that rabbits from some litters convulsed at a higher glycogen level, while those from others did so when glycogen had almost disappeared. They concluded that insulin convulsions are not necessarily related to the absence or presence of glycogen.

That low blood sugar *per se* cannot be responsible for convulsions may be readily concluded from the fact that we, as well as others, have observed that not all rabbits with sugar in the blood as low as 0.048 per cent or even 0.035 per cent develop that condition. Puttner (17) reported that 36 per cent of his animals given insulin intravenously had convulsions at a blood sugar level of 40 to 50 mgm., 32 per cent at a level of 50 to 60 mgm., 15 per cent at a level of 60 to 70 mgm., while 6 per cent died in convulsions when the blood sugar was not below 70 mgm. Bodansky (18) has recently reported in his experiments on sheep the development of hypoglycemia with insulin, but no convulsions. On the other hand we cannot ignore the fact that rabbits convulsing as a result of insulin recover rapidly after the subcutaneous administration of glucose.

That convulsions have no intrinsic relationship to blood sugar content

SERIES II

Diet: Alfalfa

Experiment 1. Weight of rabbit, 1900 grams. Insulin injected, 10 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.110	6,568,000	6,800
Time after injection:			
1 hour 30 minutes	0.066	6,784,000	7,145
3 hours 10 minutes	0.054	7,256,000	8,624

Remarks: Convulsions 3 hours 30 minutes after injection

Experiment 2. Weight of rabbit, 2300 grams. Insulin injected, 15 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.124	7,136,000	8,050
Time after injection:			
1 hour 30 minutes	0.066	7,376,000	8,620
2 hours	0.048	7,600,000	8,400

Remarks: Convulsions 5 hours after injection

Experiment 3. Weight of rabbit, 2200 grams. Insulin injected, 20 units

	BLOOD SUGAR	RED CELLS	WHITE CELLS
	per cent		
Normal	0.098	6,040,000	7,350
Time after injection:			
2 hours	0.060	6,232,000	7,560
3 hours 15 minutes	0.048	6,504,000	9,350

Remarks: Convulsions 3 hours 30 minutes after injection

may also be seen from the two experiments following. Ammonium carbonate and picrotoxin both produce convulsions, although blood sugar at the time of the onset is increased most markedly.

Convulsions with marked hyperglycemia

1. Weight of rabbit: 1600 grams.
Dose: 4.5 cc. (0.65 gram) of 14.3 per cent solution of ammonium carbonate.
Time of injection: 11:15 a.m.
Blood sugar: Normal, 0.098 per cent; 45 minutes after injection, 0.167 per cent.
Remarks: Animal hyperexcitable and in a convulsive state. Respiration and heart rapid. Glucose (20 cc. of 20 per cent solution) administered at 12 and repeated at 12:15. Injection of glucose did not allay toxic effects. Animal finally recovered.

2. Weight of rabbit: 2450 grams.
Dose: 2.4 cc. (0.48 mgm.) of picrotoxin injected subcutaneously.
Time of injection: 12:30 p.m.
Blood sugar: Normal, 0.098 per cent; 30 minutes after injection, 0.180.
Remarks: Violent convulsions at 1:00 p.m.; death at 1:20 p.m.

The high blood sugars obtained with the ammonium carbonate and with picrotoxin is probably the result of rapid glycogenolysis through excessive muscular movement and through impediment of respiration resulting from asphyxia. Macleod (19) points out the fact that a perceptible rise in blood sugar is observed immediately following insulin convulsions.

Our experiments lead us to believe that convulsions after insulin hinge upon a diet inadequate in certain respects. Our rabbits were kept on a diet made up either of 1, alfalfa, or 2, oats and fresh cabbage. On the latter regime no rabbit in our laboratory ever got into the convulsive state either on small doses of insulin obtained from the Eli Lilly Laboratories through the courtesy of Dr. G. H. A. Clowes, or from the Connaught Laboratories through the kindness of Doctors Best and Scott. Even fasting longer than twenty-four hours previous to insulin injection—two to four days—failed to bring on convulsions.

Our rabbits fed alfalfa hay invariably fell into convulsions after the injection of insulin. Blatherwick, Long, Bell, Maxwell and Hill (15), fed one set of rabbits alfalfa and another set alfalfa and crushed barley. Alfalfa is base-forming, while a mixture of alfalfa and crushed barley is acid-forming. On the former diet convulsions were observed, but not on the latter. Abderhalden and Wertheimer (20) were also able to bring about convulsions with the base-forming food, alfalfa, but not with the acid-forming substance, oats. The tendency of the investigators mentioned is to attribute inability to induce convulsions to the potential acidity of the diet. Page (21) finds that rabbits on base-forming diets show a lower fall in blood sugar after insulin injection, than those on acid diet. Bricker (22) reports that a hypolycemia is produced in rabbit blood by an introduction of an excess of hydroxyl ions, and conversely that a hyperglycemia follows an excess of hydrogen ions. Perhaps any factor that will aid glycogenolysis, such as acidosis, will tend to render the organism susceptible to the toxic effects of insulin.

That toxicity is related to diet is well known. Thus, Salant and Swanson (23) found that the resistance of rabbits to sodium tartarate could be markedly increased or decreased by dietary changes. Levine and Hubenka (24) observed that sodium salicylate is much more toxic to rabbits on alfalfa than those on oats and fresh cabbage. We maintain that variation in the toxic manifestations of insulin and other substances are directly related to the greater or lesser adequacy of the diet under observation. In the case of insulin the tendency to convulsions on certain foods may be attributed to the fact that animals on faulty diet undergo marked pathological changes in the nervous system. That inadequate nutrition is conducive to alterations in the nervous mechanism may be seen from a study of diseases, the etiology of which lies in malnutrition, such as beri-beri (25), scurvy (26) or pellagra (27). We observed con-

vulsions with alfalfa but not with oats and cabbage, although Abderhalden and Wertheimer induced this condition with oats alone. Blatherwick and his associates brought about the convulsive state with alfalfa, but not with alfalfa and crushed barley. Alfalfa or oats alone is quite inadequate. However, alfalfa supplemented with barley or oats supplemented with cabbage, is, indeed, more adequate. A mixture of leaf and seed, is, according to McCollum, Simmonds and Parsons, able more or less to supplement the deficiencies of one another.

From a clinical standpoint it is important to study further the relation of diet to insulin. Why some patients prove very refractory to insulin treatment may prove to be a problem of diet. Perhaps the exact amount of carbohydrate that may be utilized by a definite quantity of insulin depends upon some one or more dietary factors.

It is generally assumed that some definite relation exists between the irritability of the neuromuscular mechanism and the calcium and phosphorus content of the blood. A calcium-phosphorus imbalance produces marked nervous instability. Tomasson (28) determined the serum calcium in 200 inmates of an insane asylum. Eighty per cent of them gave abnormal figures for calcium. The abnormalities were found frequently in patients suffering from manic-depressive insanity.

In case of rickets the retention of calcium is very low or there may be an actual loss. In convalescence, however, the calcium retention may be two or three times the normal. In active rickets the inorganic phosphorus of the serum appears to be regularly reduced. In infantile scurvy Bahrdt and Edelstein (29) found the bone marrow and also the muscles poor in solids, calcium and phosphorus.

Tetany is said to be associated with a low calcium, which disturbs the calcium-phosphorus balance. It is interesting to note that in rickets as well as in osteomalacia, tetany may be a secondary manifestation as a result of the disturbance in the relation of calcium to phosphorus. The clinical evidence of rickets may be very slight, yet the nervous symptoms may be very marked. To prove that some nutritional defect is at the bottom of a condition of nervous instability we may merely mention the findings of Lust (30), that tetany occurs in only 2 per cent of breast fed, but in 55.7 per cent of children fed artificially.

The effect of insulin on the chemical blood picture has not yet been extensively studied. According to Briggs, Koechig, Doisy and Weber (31), insulin causes a decrease in the concentration of glucose, inorganic phosphorus and potassium in the blood. Sjollema and Seekles (32) have already observed that calcium chloride is antagonistic to the action of insulin. Perhaps some endocrine organ like the parathyroid may be involved in the mechanism of insulin convulsions. In this connection it is interesting to note that Winter and Smith (33) and also Forrest (34)

observed that parathyroid given together with insulin enhanced the action of the latter. Duchenau (35), Burn and Marks (36), and Houssay and Busso (37) found in thyroidectomized rabbits greater susceptibility to the effects of insulin and a more pronounced fall in the blood sugar after its administration.

It is now a well-established fact that faulty food affects profoundly the glands of internal secretion (38). McCarrison and others (39) have emphasized the deleterious results of improper food on such glands as the thyroid and the suprarenal capsule. Certain forms of goiter have now come to be looked upon as a deficiency disease caused by lack of iodine. According to a host of workers (40) food inadequacies bring about atrophy of the thymus. Ethel M. Luce (41) has been able to demonstrate, as a result of calcium deficiency, a progressive enlargement of the parathyroid gland due to hyperplasia. Parathyroidectomy brings about a decrease in blood calcium. Leites (42) has recently demonstrated that thymectomy also gives a lowered blood calcium. In view of the damage done to the endocrines, it would not be at all surprising to find that food faults increase the toxic manifestations of insulin.

The effect of insulin on the nervous mechanism has been studied by few. Garrelon and Santenoise (43) find this hormone a powerful excitant of the vagus. Winter and Smith (44) report that insulin acts as a depressor of the nervous cells or endings of the vagal system and not as a stimulant to the inhibitory sympathetic endings. Insulin slows cardiac action and respiratory activities. Blatherwick, and his associates (15) observe that convulsions are more easily produced after rabbits have had one or more attacks. This fact shows that the nervous mechanism, already irritated, succumbs more easily to a second or third stimulation. Garrelon and Santenoise (43) believe that insulin increases susceptibility to shock.

That nutrition itself may be an important factor in the utilization and in the toxic manifestations of insulin, is by no means improbable. Some clinical facts point in this direction. Bodansky and Simpson (45) have observed that insulin hypoglycemia is not as intense in fat as in lean subjects. Joslin, Gray and Root (46) have also noticed that children and adults, weakened and desiccated especially by diarrhea, are prone to develop dangerous hypoglycemia even with small amounts of insulin. Abderhalden and Wertheimer (47) have shown that rats fed a carbohydrate-free diet reacted less violently than those on a mixed diet of carbohydrate, fat and protein. The beneficial results of glucose on insulin hypoglycemia do not harmonize with the findings of the German workers with reference to a carbohydrate-free diet. Very recently Voegtlin, Dunn and Thompson (48) stated that the mode of action of insulin not only involved carbohydrate but also protein and fats. They found that when given by mouth, the carbohydrates, glucose, fructose, maltose,

lactose, sucrose and trehalose, are physiological antagonists of insulin. They also found glycerol very effective as a preventive of insulin death and as an agent useful in recovery from hypoglycemia. The amino acid, alanine, proved very useful in about the same amount as glucose in decreasing the hypoglycemia following insulin.

With reference to diet in diabetes, clinicians have thus far focused their attention upon glucose intake from carbohydrate, fat and protein, and upon fat and glucose in relation to ketogenesis. Little or no emphasis has been laid on the necessity for vitamins and for inorganic components like iodine, iron, calcium and phosphorus. Since diabetes usually takes a chronic course, it may be readily seen how a restricted and inadequate diet continued for a long period may result in the development of symptoms of a full-fledged or borderline deficiency disease with a resultant lowering of the resistance of the patient to combat successfully with his primary condition.

That restriction in diet does lead to serious disaster is seen from the fact that a typical case of beri-beri has been discovered near Omaha in a women of about thirty. Because of diabetes she had been advised a very restricted diet. After having lived on such diet for over six years, she was finally referred to Dr. M. G. Wohl. In addition to diabetes one of us (Levine) diagnosed the case as one of typical beri-beri. We may say in reference to this patient that the remedy proved worse than the malady. A detailed account is being prepared for publication.

The cause of diabetes has been laid largely at the door of the pancreas. Perhaps other organs, like the liver, are also accomplices. Faulty food is known to bring about pathological changes in the pancreas and in the liver. Among the many important contributions of McCarrison (49), one lies in the fact that chronic lack of vitamin B in the food leads to atrophy of the cells upon whose functional perfection the normal metabolism of carbohydrate depends. A diet deficient in vitamin B brings about atrophy of the pancreas. Funk (50) observed in experimental beri-beri an increase in blood sugar and a lowered tolerance. Kozawa and associates (51) and also Hosokawa (52) reported similar results. Funk and his associates (53) observed that a large addition of carbohydrate to the diet increased the vitamin B requirement of the organism. Rondoni and Montagnani (54) reported in vitamin C deficiency that the degenerative changes in the thyroid and in the adrenals go hand in hand with hypertrophy of the islands of Langerhans. Palladin (55) demonstrated a hyperglycemia in guinea pigs on a scorbutic diet. This hyperglycemia, however, was followed later by hypoglycemia. Changes in the diastase content of the blood paralleled those in the sugar content, and the glycogen of the liver showed a decrease. Should an incomplete diet be excessively rich in carbohydrate, one can readily see how the islands of

Langerhans would all the more easily fatigue as a result of overload. Furthermore, such an inadequate and one-sided diet would give rise to degenerative processes in glands of internal secretion other than the pancreas. With the endocrine system as a whole under-functioning, food would go to fat, especially under the conditions of a sedentary life.

It seems from our present state of knowledge that the etiology of diabetes rests upon a tripod, the individual legs of which are 1, lack of vitamin in the diet; 2, excessive carbohydrate intake; and 3, a sedentary life. That these three facts fit into actual human experience may be seen in India among the rice-eating Hindus, and among the Jews. McCarrison (56) observes that diabetes is very common among the rice-eating classes of India, if their habits are sedentary, and especially if they eat largely of sweet-meats. Joslin (57) and others have pointed out the great incidence of diabetes among Jews, especially those suffering from overweight. Anyone at all familiar with Jewish diabetics will at once recognize that they are drawn largely from the rank of those who lead a sedentary life, and whose diet is exceptionally overdone in carbohydrate and extremely poor in fresh fruit and vegetables. We may describe this diet, in the language of McCollum (58), as a bread-meat-cereal-potato diet, poor in dairy and plant products. It is apropos to mention here that Desgrez, Bierry and Rathery (59) have tried out the effects of vitamin B on the diabetic under insulin treatment. They state that the result of the addition of vitamin B is to enhance and to prolong the effect of the insulin injection and to permit of a greater spacing of time between the doses.

SUMMARY

The subcutaneous injection of insulin causes an increase in the number of erythrocytes and in the number of leucocytes per cubic millimeter of blood. The increase in both the red and the white cells is suggestive of the existence of a state of anhydremia.

No relation exists between the size of the insulin dose and the increase in the cell components of the blood. The rise in these components, however, has a definite bearing on the fall in blood sugar. The lower the blood sugar falls, the greater becomes the increase in the red and white count.

Convulsions after insulin administration depend on diet, not upon an existing state of hypoglycemia. This finding confirms that of Blatherwick, Long, Bell, Maxwell and Hill, and also that of Abderhalden and Wertheimer.

It is suggested that convulsions in an animal given insulin bring to light a state of inadequate nutrition, which is known to cause nervous instability. This instability reveals itself especially in the presence of so vigorous a pharmacological agent as insulin.

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